QH 1
C192
v.8
Copy 2

The Effect of Fin-Clipping on the Cruising Speed of Goldfish and Coho Salmon Fry

By Roland W. Radcliffe
Department of Zoology, University of British Columbia
(*Received for publication August 15, 1949*)

ABSTRACT

Goldfish (*Carassius auratus L.*) and coho salmon fry (*Oncorhynchus kisutch* Walbaum) were acclimatized to temperatures of 20° C. and 3° C. respectively. The fish were placed, one at a time, once a day, for ten days, in a rotating annular chamber and the cruising speed was found. Then various fins were removed and the fish were given ten more trials. The mean cruising speeds before and after clipping were compared. The clipped fish suffered no loss in ability to swim at a constant rate. The data suggest that, for fish of a given weight and length, cruising speed is slightly improved by clipping. Any increased mortality in marked fish is due to loss of stability and control rather than loss in ability to swim steadily.

The removal of one or more fins from a fish is a standard method of marking them for future identification. Such procedures are now routine in fisheries investigations and are commonly resorted to in determinations of population size, mortality rates, and migration routes. Any mortality caused by the marking procedure will produce inaccuracies in the analysis of the results unless adequate adjustments are made.

Two sources of marking mortality may be anticipated. First, there may be death from rough handling or subsequent infection of wounds. Second, there may be a loss of the fish's ability to maintain its equilibrium, to swim or to manoeuver so that it is more easily caught by predators or is not able to catch its own food. Death from the first cause is readily checked by experiments in which fish are placed in a pond free of predators and their mortalities compared with control groups. The results may be expected to vary with the species of fish but the mortality from this cause is usually negligible (Ricker, 1949). Differential death from this cause cannot be readily determined while the fish are in captivity.

The experiments undertaken in this study were designed to test the effect of fin-clipping on the cruising speed of fish. The "cruising speed" has been defined as "that speed at which the fish could swim steadily for some considerable period of time, although presumably after hours fatigue would set in" (Fry and Hart, 1948). This speed is used here as a measure of the activity of the fish.

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

ACKNOWLEDGEMENTS

These data are from a thesis presented in partial fulfilment of the requirements for the degree of Master of Arts in Zoology, University of British Columbia.

The writer is indebted to Dr. E. C. Black who suggested the problem and loaned the cruising speed apparatus, and to Dr. W. S. Hoar who supervised the research and assisted in the preparation of this manuscript.

APPARATUS AND METHODS

The cruising speed was measured by means of a rotating annular chamber similar to the one used by Fry and Hart (1948). When the chamber was rotated the fish swam in a direction opposite to the rotation of the drum and maintained a fixed position relative to the room. A lamp was placed on one side of the rotating chamber five centimeters from the outer wall in the mid line to aid the animal in orienting itself. This pseudo-rheotropic response was elicited mainly by the short radius of curvature of the tank and to a lesser extent by the optical stimulus of the light (Gray, 1937). A thermometer suspended in the water may have acted as an additional stimulus.

The ratio of the speed of the drum to the speed of the water over the range used for the experiments was constant, so speeds will be stated in revolutions per minute, since it is the relative speeds before and after clipping that are of interest and not the absolute values.

The procedure followed in testing a fish was as follows: the fish was put in the chamber which was rotated at a slow speed for 12 minutes. The microcontrol of the reduction gear was set at 1.5 which gave a speed of approximately 20 r.p.m. for this initial period. No record was kept if laps were lost during the initial orientation time. After 12 minutes the speed was increased by 0.5 units of the microcontrol (approximately 8 r.p.m.) and each succeeding two minutes it was increased by the same amount until the fish began to lose laps. Then, the speed was reduced by 0.1 units at two minute intervals until the fish could just maintain its position. The number of revolutions was now counted using a stop watch and this was taken as the cruising speed. Each trial lasted from 25 to 30 minutes.

The goldfish were obtained from the Goldfish Supply Co., Stouffville, Ontario and acclimatized to a temperature of 20°C. for three weeks before being used for the experiments. They were fed dessicated liver and pablum. The water used to make the trials was also kept at 20°C. Each fish was given three preliminary trials, one day apart. Then each fish was tested once per day for ten days. Following this fins were cut off in various combinations (tables I and II).

Four fish were marked for each combination. The clipped fish were then given one trial each day for another ten-day-period and the mean cruising speeds of each fish before and after being clipped were compared.

The coho fry were treated in the same manner except that they were kept in a trough of running water at 3°C. and were rotated in water at 5°C. They were fed frozen liver. Two coho were marked for each combination.

Control fish of both species were given daily trials for the second ten-day period, and the mean cruising speeds were compared as in the case of clipped fish.

The fin combinations were those commonly used for marking on the Pacific Coast: anal and right ventral, dorsal and left ventral, dorsal and right ventral, both ventrals, dorsal and both ventrals, anal and right pectoral.

TABLE 1—Cruising speed of goldfish before and after marking. A—anal; B—both; D—dorsal; L—left; P—pectoral; R—right; V—ventral; $\bar{x}_1$ and $\bar{x}_2$—mean cruising speeds before and after fin-clipping (10 trials).

Weight gm.	Type of Marking	Mean before Marking ($\bar{x}_1$) Ten Trials	Change in Cruising Speed $\bar{x}_1 - \bar{x}_2$	$s_{\bar{x}}$	t
2.0	ARV	36.02 r.p.m.	−2.12	5.54	0.383
5.5	ARV	79.11	8.50	11.20	0.758
3.4	ARV	52.08	−7.38	8.34	0.886
5.1	ARV	59.71	3.97	8.64	0.459
5.2	DLV	51.28	−12.32	8.64	1.430
5.1	DLV	57.68	−2.60	8.70	0.298
7.1	DLV	66.61	1.48	9.74	0.148
5.9	DLV	58.21	5.15	8.33	0.618
4.9	DRV	54.74	1.48	8.50	0.174
6.6	DRV	78.87	6.75	11.29	0.599
4.6	DRV	55.02	1.09	8.11	0.134
8.6	DRV	80.76	2.87	11.6	0.246
7.9	BV	55.45	−15.87	9.51	1.68
7.7	BV	62.16	3.80	8.99	0.423
8.0	BV	59.53	−2.07	9.04	0.229
2.2	BV	48.50	−2.43	7.41	0.328
8.3	DBV	57.49	2.00	8.41	0.238
6.8	DBV	60.51	11.22	8.22	1.361
4.6	DBV	65.59	6.10	9.34	0.654
11.1	DBV	58.04	−2.93	8.86	0.331
13.4	ARP	71.27	2.30	10.45	0.220
6.3	ARP	73.10	−2.35	11.05	0.212
13.8	ARP	59.61	−3.73	9.20	0.405
13.8	ARP	68.42	4.13	9.90	0.418
11.9	Control	74.28	7.74	10.51	0.774
11.8	Control	67.28	−1.34	10.15	0.132
12.2	Control	62.58	−13.51	10.40	1.357
11.5	Control	58.07	−1.10	8.75	0.125
15.6	Control	63.48	−16.87	10.70	1.565
13.8	Control	57.00	−7.58	9.10	0.834

*All statistical symbols from Snedecor, G. Statistical Methods, 1846.

RESULTS

The data are summarized in tables I and II. The t values given in these tables show that there is no difference in the mean cruising speed of marked and unmarked fish. Table III shows the day to day unaccountable variation in cruising speed which was true of both the goldfish and the coho. There is a suggestion that fish may be able to swim slightly better after their fins are removed. This is seen in an analysis which was made to demontrate relationships between size of goldfish and their cruising speed (table IV). In this table it will be noted that there is a positive correlation for both weight and length with the cruising speed and that the correlation is slightly more significant after clipping than before the mutilation.

TABLE II. The comparison of the mean cruising speeds before and after marking coho fry. The same abbreviations are used as in Table I.

Fish No.	Weight gm.	Mean before Marking ($\bar{x}_1$) Ten Trials	Mean after Marking ($\bar{x}_2$) Ten Trials	Type of Marking	$\bar{x}_1 - \bar{x}_2$	$s_{\bar{x}}$	t
33	5.4	66.28 r.p.m.	64.44 r.p.m.	BV	1.84	9.75	0.188
34	9.8	65.65	59.42	BV	6.23	9.34	0.624
35	7.3	42.37	51.15	DRV	−8.78	7.00	1.250
36	8.5	61.43	62.88	DRV	−1.45	9.25	0.156
31	8.8	69.29	69.55	Control	−0.26	7.32	0.035
32	7.1	52.23	49.17	Control	3.06	7.55	0.406

TABLE III. Typical protocol of the maximum cruising speed of a goldfish for each trial. Fish number 9, 5.2 gm., 5.7 cm.

Trial	Before Clipping	Trial	After Clipping DLV
1	48.9	1	52.6
2	46.7	2	63.8
3	44.8	3	58.9
4	50.0	4	60.0
5	51.7	5	81.0
6	46.2	6	66.6
7	52.6	7	68.2
8	65.4	8	60.0
9	50.0	9	65.4
10	56.5	10	59.5

TABLE IV. Summary of statistical data showing correlations of cruising-speed and weight, cruising-speed and length of goldfish.

	Before Clipping			After Clipping		
	b	95% Fiducial Limits	t	b	95% Fiducial Limits	t
Cruising-speed and weight	1.04	2.02 to 0.06	2.16	1.84	2.50 to 0.98	3.71
Cruising-speed and length	3.38	6.29 to 0.47	2.38	4.90	7.60 to 2.20	3.57

DISCUSSION

In these experiments an attempt has been made to discover whether a fish is at a disadvantage in a steady swimming test when various fins have been removed. Such a disadvantage might be correlated with the animal's ability to avoid enemies or capture food. Foerster (1936) found an extra mortality of approximately 62% in the ocean among marked sockeye salmon migrants (*Oncorhynchus nerka*). The analysis of this experiment has been criticized (Ward, 1939) but Ricker's (1949) recent paper shows that for certain species of fish the removal of fins does have an adverse effect on the animal's ability to adapt itself to its environment. This, however, was not true for all species under the conditions of the experiment.

The present experiments show that this disadvantage, if it exists in goldfish and coho fingerlings, cannot be attributed to the impairment in swimming ability. The analysis of the mean cruising speed by the *t* test of significance clearly shows that the clipping of two fins has no effect on the cruising speed of either goldfish or coho fry. Their ability to swim at a steady speed for a considerable time is not affected. Some of the mean speeds after the clipping were higher but this was not consistently the case. Further, the differences in the controls were of the same order as the experimental fish.

The standard deviations indicate a considerable variability in the cruising speed of each trial. Over a period of twenty trials a constant cruising speed was never obtained. A fish would show a fairly consistent speed for a few days and then it would change radically for no apparent reason. No fish showed constancy for the whole twenty trials. The time of day or the time of trial relative to feeding seemed to make no difference, neither did the relative number of the trials nor the day of the month appear to be correlated with the changes.

There are two possible explanations of the negative results: either the apparatus was inadequate or the mutilation had no effect on steady swimming.

Considering the first possibility, the fish may not be stimulated to expend a maximum of energy at any given trial. However, the condition of light, temperature and the method of increasing the speed were kept constant in all trials, so it can be assumed that the fish were equally stimulated for each trial.

The results of this study are in accord with Gray's (1936) analysis of fish locomotion. He says that the movement of a fish through the water is caused by the contraction waves passing alternately down each side of the body. If the caudal fin is removed the fish compensates by increasing the oscillation of the posterior part of the body and by moving a larger part of the body. Whether fatigue sets in sooner in such a fish is not stated.

Clipping the fins will reduce the weight of a fish a slight amount and also remove some of the drag, providing the fin is amputated close to the body. Hence, it is conceivable, from theoretical considerations, that an increase in cruising speed could result from clipping the fins. The data of table IV are pertinent in connection with this theory. The regression coefficients, "b", increase slightly after clipping and suggest that the cruising speed may be somewhat improved by the operation. At the same time, the increase in "t" value indicates an improved correlation, after the clipping, between body size and swimming. Individual variations in ability of fish to swim seem to be related to the use of the fins and one can more accurately predict speed from weight or length after removing the fins.

Removal of fins probably has a more serious effect on the equilibrium of a fish than on its ability to swim. Equilibrium effects are greatest when the animal is turning, accelerating or decelerating, and performing combinations of these manoeuvres. Harris (1936, 1937, 1938) has shown experimentally and theoretically the function of the fins in maintaining equilibrium and the consequences of cutting off certain fins. The experiments were done by making a plaster cast of the fish with adjustable wax fins and putting the model in a wind tunnel. Harris (1936) has also shown that fish do not learn to compensate in a period of two months for the loss of their clipped fins. His analysis clearly demonstrates the important role of the fins in stability and control. It seems probable that this factor plays a large part in any increased mortality of marked fish but that the fish's ability to swim at a steady speed is little affected by the removal of its fins.

REFERENCES

FOERSTER, R. E. The return from the sea of sockeye salmon (*Oncorhynchus nerka*) with special reference to percentage survival, sex proportions and progress of migration. *J. biol. Bd. Canad.*, **3**, 26-42, 1936.

FRY, F. E. J. AND J. S. HART. Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd. Canad.*, **7**, 169-175, 1948.

GRAY, J. Studies in animal locomotion. III. The propulsive mechanism of the whiting (*Gadus merlangus*). *J. exp. Biol.*, **10**, 391-400, 1933.

Pseudo-rheotropism in fishes. *J. exp. Biol.*, **14**, 95-103, 1937.

HARRIS, J. E. The role of fins in the equilibrium of the swimming fish. I. Wind-tunnel tests on a model of *Mustelus canis* (Mitchill). *J. exp. Biol.*, **13**, 476-493, 1936.

The mechanical significance of the position and movements of the paired fins in the Teleostei. *Pap. Tortugas Lab. Carn. Instn. Wash.*, **31**, 173-189, 1937.

The role of fins in the equilibrium of the swimming fish. II. The role of the pelvic fins. *J. exp. Biol.*, **15**, 32-47, 1938.

RICKER, W. E. Effect of removal of fins upon the growth and survival of spiny-rayed fishes. *J. Wild. Mgt.*, **13**, 29-40, 1949.

SNEDECOR, G. W. Statistical methods. 4th. ed. Ames, Iowa State College Press. 1946.

WARD, H. B. Factors controlling salmon migration. *Amer. Assoc. Adv. Sci. Pub.*, **8**, 60-71, 1939.

The Maillard Reaction in Fish Products

By H. L. A. Tarr
Pacific Fisheries Experimental Station,
Vancouver, B.C.

(*Received for publication, August 19, 1949*)

ABSTRACT

The brown discolouration which occurs on heating (120°C., 1 hr.) white flesh of fish was found to be due largely to reactions of the Maillard type. Browning did not occur in leached flesh unless compounds containing a free aldehyde group, or a potential free aldehyde group, were added. Addition of amino acids and substances which did not possess a free aldehyde group did not occasion browning in leached flesh. Discolouration was inhibited by bisulphite and hydroxylamine and slightly by acids, but it was not affected by heating in vacuo, by storing whole fish in ice prior to heating or by addition of metal ions.

Maillard (1912) first drew attention to the fact that the free aldehyde group of glucose reacted with the amino group of amino acids to yield brown condensation products (melanoidins). The significance of this discovery, which was destined to become of great interest to food chemists, was not recognized for many years. Enders (1938) investigated the chemistry of the reaction both with regard to condensation between isolated aldehydes and amino acids and to its importance in determining desirable and undesirable colour and flavour alterations in foods and beverages. He suggested that initially there was formed an N-glycoside linkage between aldehyde groups and amino groups, and obtained some evidence in support of his contention that pyrrol-like brown substances were ultimately produced when reducing sugars condensed with amino acids. Compounds which yielded free sulphur dioxide inhibited the reaction, presumably by combining with and inactivating aldehyde groups. Enders showed that diacetyl, glyceraldehyde, dihydroxyacetone and acetaldehyde all reacted with amino groups and suggested that reductone, which forms during alkaline degradation of glucose, might play an important role in the industrial occurrence of the Maillard reaction. There does not appear to have been any outstanding advance in the knowledge of the basic chemistry of the reaction since the appearance of Ender's paper.

During World War II deterioration in stored dehydrated and other foods became an acute problem, and it was soon realized that the Maillard reaction played an important part in determining loss of quality. It became known as the "Industrial Browning" or "Sugar-protein" reaction, and its occurrence and control in a variety of foods was extensively investigated (Pearce, 1945;

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

Barnes and Kaufman, 1947). It has been stated that the browning of dehydrated fish is the result of a typical Maillard reaction (Kaufman, 1946), but no experimental results in support of his contention have been published.

In view of these facts an investigation concerning the role of the Maillard reaction in non-enzymic browning of fishery products, particularly in those subject to heat processing, was initiated by the writer (Tarr, 1948). Detailed results of this work are recorded in the present paper.

EXPERIMENTAL

In general the experiments to be described were designed to determine colour changes which occurred in treated and untreated flesh of white muscled fish under conditions somewhat similar to those observed in commercial canning (processing) procedures.

Either strictly fresh fish or defrosted fish of good quality were used. They were filleted and the fillets were minced. Leaching of the minced flesh was accomplished by suspending it ten successive times in large volumes of cold tap water and decanting the supernatant liquid. It was finally suspended in distilled water and the free liquid was removed by placing it on conical filters and draining overnight at 0°C.

The purest grades of organic compounds obtainable were used. The sources of some which were found difficult to obtain and methods of preparation of others were as follows: reductic acid, reductone and sodium dihydroxymaleate (Tarr and Cooke, 1949); glucose-1-phosphate (di-potassium dihydrate), dihydroxyacetone and sodium-3-glycerophosphate (A.D. Mackay, New York); sodium *l*-ascorbate and sodium d-iso ascorbate (Hoffmann-La Roche); glyceraldehyde (Schering-Kahlbaum); pyruvaldehyde (30.4% aqueous solution), (Carbide and Chemicals Corporation, New York); pyruvaldehyde (polymerized substance prepared from the foregoing solution by the method of Harries and Türk, 1905) and synthetic barium glucose-6-phosphate (Levene and Raymond, 1931).

Unless otherwise stated samples were heated (processed) for 1 hour at 120°C. (15 lb. steam pressure).

The colour intensity was determined with an Armstrong colorimeter (Charnley 1936), an instrument designed specifically for use in the local Dominion Canned Fish Inspection Laboratory in determining the red and yellow colour of canned salmon. Colour of the flesh could be matched fairly closely by superimposing red, yellow and blue Lovibond colour glasses. In order to show their relative proportions red, yellow, and blue colour units have been given in only the first two tables. Though the ratio of these colours was by no means constant, yellow invariably predominated, except in one sample of dihydroxymaleate-treated flesh. Blue, which was only noticeable in the more deeply coloured samples, was invariably less intense than red. The total (red, yellow and blue) units, recorded in all tables, seemed to give a fairly good comparison with the degree of browning as measured visually.

Effect of Leaching

The pooled minced flesh of 4 small red cod (*Sebastodes* sp.) was divided equally, one portion being leached and the other left untreated. The results obtained on heating representative portions of the flesh are recorded below. They show that a much less marked discolouration occurred when the flesh was leached prior to heating.

	Colour intensity in Lovibond units			
	Red	Yellow	Blue	Total
Leached flesh	0	0.7	0	0.7
Untreated flesh	3.0	4.0	1.0	8.0

Effect of Reducing Sugars, Certain Other Organic Reducing Substances and Amino Acids

The substances listed in table I were incorporated into 25-g. portions of minced leached lingcod flesh so that they were present in 0.01 M concentration

Table I. Effect of sugars and related substances and of amino acids on browning of leached lingcod flesh.

Compound added (0.01 M concentration)	Colour intensity in Lovibond units			
	Red	Yellow	Blue	Total
None	0	0.7	0	0.7
Glycine	0	0.9	0	0.9
dl-Alanine	0	0.7	0	0.7
dl-Threonine	0	0.7	0	0.7
dl-Phenylalanine	0	0.7	0	0.7
l-Proline	0	0.6	0	0.6
l-Hydroxyproline	0	0.7	0	0.7
Trimethylamine oxide	0	0.9	0	0.9
Mannitol	0	0.8	0	0.8
Sucrose	0	0.8	0	0.8
Lactose	1.4	2.8	0	4.2
Mannose	2.7	3.4	0	6.1
Fructose	1.0	2.5	0	3.5
Galactose	2.5	4.0	0	6.5
Glucose	1.0	3.5	0	4.5
Arabinose	3.4	5.0	0.5	8.9
Xylose	3.8	5.0	0.5	9.3
Glucose-6-phosphate	2.0	3.6	0.3	5.9
Sodium Dihydroxymaleate	3.0	3.0	2.4	8.4
Glyceraldehyde	2.0	3.4	0.6	6.0

in the samples. The samples were heated in 100 ml. covered glass beakers, and the results are recorded in table I. The amino acids, sucrose, mannitol and trimethylamine oxide did not cause noticeable browning; on the contrary the reducing sugars, particularly the aldo-pentoses, and glyceraldehyde, glucose-6-phosphate and sodium dihydroxymaleate all occasioned marked browning.

To 22.5-g. portions of minced leached lingcod flesh in 100-ml. covered beakers were added 2.5-ml. portions of 0.1 M solutions of the compounds listed in table II. The results obtained on heating the samples are recorded in this

TABLE II. Effect of reducing sugars, related substances and certain other reducing compounds on browning of leached lingcod flesh.

Compound added (0.01 M concentration)	Colour intensity in Lovibond units			
	Red	Yellow	Blue	Total
None (Leached flesh)	0.2	0.6	0	0.8
None (Unleached flesh)	1.5	2.5	0	4.0
Glucose	0.8	1.5	0	2.3
Glucose-1-phosphate	0.8	1.6	0	2.4
Glucose-6-phosphate	0.9	1.6	0	2.5
Sodium 1-ascorbate	0.3	1.0	0	1.3
Sodium d-iso ascorbate	0.3	1.0	0	1.3
Pyruvaldehyde (30.4%)	0.8	1.5	0	2.3
Pyruvaldehyde (polymerized)	0.9	1.5	0	2.4
Dihydroxyacetone	1.0	1.6	0	2.6
Glyceraldehyde	1.0	1.7	0	2.7
Reductone	1.5	1.9	0	3.4
Reductic acid	0.8	1.9	0	2.7
Sodium dihydroxymaleate	3.0	2.5	1.2	6.7
Sodium-3-glycerophosphate	0.2	0.6	0	0.8

table. They show that all the substances listed with the exception of sodium-3-glycerophosphate, which does not possess a free aldehyde group, caused browning. Also the unleached flesh browned much more markedly than that which had been leached.

EFFECT OF VACUUM

Leached and unleached samples of minced flesh of defrosted halibut (*Hippoglossus stenolepis*), brill (*Eopsetta jordani*), whitefish (*Coregonus clupeaformis*) and red cod were prepared. To samples of both leached and unleached flesh arabinose in 0.01 M concentration was added. Samples weighing about 10 g. were placed in 18 mm. diameter pyrex tubes. Some of these were covered with an aluminum cap and others were sealed after evacuating with a high vacuum pump. The results obtained before and after processing these samples in the

usual manner and heating at 100°C. for 5 minutes are given in table III. It will be seen that the colour of the raw flesh varied considerably. The flesh of

TABLE III. Effect of vacuum on browning.

Treatment	Total colour intensity in Lovibond units			
	Halibut	Sole	Red cod	Whitefish
Raw flesh	1.3	2.2	3.9	6.4
Heated 5 min. at 100°C.	1.0	1.2	1.6	2.2
Heated 1 hr. at 120°C.	3.2	8.0	8.3	2.8
Heated 1 hr. at 120°C. in vacuo	3.2	9.2	9.8	2.6
Heated 1 hr. at 120°C. with 0.01 M arabinose	8.2	12.0	23.0	3.8
Heated 1 hr. at 120°C. with 0.01 M arabinose in vacuo	8.5	12.5	19.8	2.9

the whitefish had a pronounced red colour which contributed to the high result obtained. In all samples, heating at 100°C. for 5 minutes decreased the colour considerably, while, except with whitefish, heating at 120°C. for 1 hour caused a marked increase especially in those containing arabinose. Samples heated with and without arabinose browned to about the same extent whether processed in vacuo or in the usual manner.

EFFECT OF INHIBITORS

Five ml. quantities of solutions of inhibitors of concentration calculated to add the amounts listed in table IV were incorporated into 45-g. portions of red cod flesh. The samples were processed as usual in covered 100 ml. beakers.

TABLE IV. Effect of sodium bisulphite and hydroxylamine on browning.

Compound added (Per cent)	Total colour intensity in Lovibond units
None	7.6
0.025 $NaHSO_3$	7.4
0.1 "	7.8
0.2 "	6.8
0.3 "	3.7
0.4 "	3.4
0.5 "	3.2
1.0 "	1.6
0.5 NH_2OH	1.6

The results, recorded in table IV, show that 1% sodium bisulphite inhibited colour development to the same extent as did 0.5% hydroxylamine. Low concentrations of bisulphite (0.025 and 0.1%) did not inhibit browning.

Effect of Storage

Eight rock cod (*Sebastodes* sp.) still in rigor were dressed, washed thoroughly and iced and stored in a room at 0.5°C. At the commencement of the experiment a piece of flesh weighing roughly 100g. was removed from one side of each fish, and this was repeated after the fish had been 16 days in ice and were quite stale organoleptically. The results obtained on heating the muscle samples in the usual way are recorded in table V. When Student's test was applied to the

Table V. Effect of storing rock cod in ice on browning.

Days stored	Total colour intensity in Lovibond units in fish no.									
	1	2	3	4	5	6	7	8	$\bar{x}$	S
0	4.1	2.9	2.6	5.5	2.9	3.2	5.7	4.4	3.91	1.11
16	5.6	5.1	3.0	4.8	3.6	3.3	2.9	4.5	4.10	0.96

means given in this table* it was found that there was no significant difference in the average colour intensity which developed on heating before and after storage.

Effect of pH

Five ml. quantities of H_20 or of H_20 containing 1.0 N HCl or NaOH were added to 45-g. portions of minced defrosted red cod flesh so that the pH values recorded in table VI resulted. On heating the samples it was found (table VI)

Table VI. Effect of pH values above and below 7 on browning.

pH value after heating	6.35	6.45	6.55	6.70	7.00	7.30	8.00	8.50	8.90
Total colour intensity in Lovibond units	4.7	4.8	5.3	5.3	5.7	7.3	13.0	14.6	15.5

that an increase in pH above 7 caused a marked increase in browning, while a decrease in pH between the values of 7 and 6.35 occasioned only a moderate decrease in browning.

An experiment similar to the foregoing one was performed, the pH of the flesh being adjusted to the values given in table VII with 5-ml. portions of orthophosphoric, acetic or citric acid solutions of appropriate strength. The

*I am indebted to Mr. N. E. Cooke of this laboratory for these calculations.

TABLE VII. Effect of orthophosphoric, acetic and citric acids on browning.

Acid	None	Orthophosphoric			Acetic			Citric		
pH.....	6.85	5.45	6.00	6.45	5.20	5.70	6.30	5.35	5.95	6.50
Total colour intensity	6.9	6.2	6.0	6.4	6.2	6.3	6.5	6.4	6.4	6.4

results (table VII) show that acidification of the flesh, even to values below 6 where considerable heat coagulation of the muscle proteins was evident, decreased browning but slightly.

EFFECT OF METAL IONS

Leached lingcod flesh (22.5g.) was placed in each of a number of 100 ml. covered glass beakers. To each portion of flesh was added 2.5 ml. of water containing (1) glucose to make an 0.01 M concentration in the flesh and (2) sufficient quantities of the following salts to make 1 and 5 parts per million concentrations of metal ions: aluminium chloride, cobaltous chloride, cupric chloride, ferric chloride, ferrous sulphate, nickel chloride, silver nitrate, stannous chloride and zinc chloride. On heating, a very slight decrease in browning was observed with the iron salts and with 5 parts per million of copper, while the remaining metallic ions were without effect.

DISCUSSION

It has been shown that the browning which occurs when the white flesh of fish is heated at 120°C. for 1 hour can be largely overcome if it is leached in water prior to such processing. Addition of reducing sugars, or of organic compounds containing a free aldehyde group or potential aldehyde group, occasioned browning of leached flesh, while addition of amino acids, sucrose or compounds which lacked a free aldehyde group was without effect. The addition of sodium bisulphite or hydroxylamine prior to heating prevented or decreased browning, and it was somewhat less severe in samples in which the natural pH of the flesh had been lowered prior to processing. Heating in vacuo did not affect the intensity of discolouration. Thus in all respects the browning of heated white flesh of fish appeared to be due to reactions of the Maillard type.

The exact nature of the substances which react in fish flesh to cause browning remains to be determined. It has been shown that fish muscle contains fructose and glucose phosphates and other organic compounds which form a potential source of reducing pentoses (Tarr, submitted for publication 1949). However, these tend to decrease in concentration when muscle is kept at 0°C., and browning has been shown to be not significantly affected by storing fish in ice for sixteen days. Thus it would appear unlikely that the degree of browning in fish flesh is controlled entirely by these reducing substances. It has been observed that the intensity of the brown discolouration varies greatly with different fish,

though whether this difference is due to species, seasonal or other variations is not yet known. The susceptibility to browning may be determined more by variations in the flesh constituents which possess free amino groups than by the nature and concentration of aldehydic substances.

Undoubtedly the occurrence of non-enzymic browning in fish products is of considerable economic importance. Thus samples of canned white fleshed fish are not infrequently discoloured and possess a faint caramelized aroma. Attention has been drawn to the fact that the tomato sauce in canned herring is often discoloured and that the flesh has a rather bitter or cooked flavour (Dreosti, 1947). As has already been pointed out (Tarr, 1948) there is little doubt that these alterations are brought about by Maillard reactions during processing. It may well be that the browning which sometimes occurs in dehydrated and salted fish results from reactions of this type. The possibility that amino-aldehyde reactions contribute in bringing about the quality improvements or deteriorations sometimes observed in canned fish products which have been stored for considerable periods must not be discounted.

At present few control measures are available. Leaching of raw flesh seems to occasion considerable loss of flavour, but removal of exuded liquid from cooked flesh of halibut has been found to yield an improved product (Borg, 1946). The present work indicates that acidification of the flesh reduces browning only slightly and would therefore seem of limited value as a preventive measure. The addition to fish flesh of sulphur dioxide, or of compounds which liberate this readily, would hardly seem desirable. In this connection it is of interest that Shewan (1948) has obtained evidence which indicates that in salted sea fish added sodium bisulphite combines with formaldehyde formed during the breakdown of trimethylamine oxide to dimethylamine. The writer has found that when sodium bisulphite is added to lingcod flesh in 0.025 to 1.0% concentration a considerable, though variable, percentage can be recovered after heat processing. This indicates that treatment of fish prior to canning with sulphur dioxide, bisulphites or hydrosulphites, as recently proposed by Mathiesen, (1949) might yield canned products containing some residual sulphur dioxide.

REFERENCES

BARNES, H. M. AND C. W. KAUFMAN. *Ind. Eng. Chem.* **39,** 1167-1170, 1947.

BORG, E. M. U.S. Patent 2,411,188, Nov. 19, 1946.

CHARNLEY, F. *Biol. Bd. Can. Prog. Rep. Pac.*, **29,** 12-16, 1936.

DREOSTI, G. M. Fishing Ind. Res. Inst. (South Africa) Bull. No. 1, 1-13, 1947.

ENDERS, C. *Kolloid Zeit.*, **85,** 74-87, 1938.

HARRIES, C. AND H. TÜRK. *Ber.*, **38,** 1630-1636, 1905.

KAUFMAN, C. W. *Food Ind.*, **18,** 80-83, 1946.

LEVENE, P. A. AND A. L. RAYMOND. *Jour. Biol. Chem.*, **92,** 757-763, 1931.

MAILLARD, L. C. *Compt. Rend.*, **54,** 66-68, 1912.

MATHIESEN, E. U.S. Patent 2,461,651, Feb. 15, 1949.

PEARCE, J. A. *Food in Canada* **5,** 24-35, 1945.

SHEWAN, J. M. *Analyst*, **73,** 605-607, 1948.

TARR, H. L. A. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **74,** 17-18, 1948. *J. Fish. Res. Bd. Can.*, (Submitted for publication 1949).

TARR, H. L. A. AND N. E. COOKE. *J. Fish. Res. Bd. Can.*, **7,** 522-527, 1949.

The Physical Limnology of Lakelse Lake, British Columbia

By J. R. Brett
Pacific Biological Station, Nanaino, B.C.

(*Received for publication, September 20, 1949*)

ABSTRACT

Lakelse lake is a temperate, eutrophic lake situated on the eastern margin of the Coast Range mountains. For five years physical and chemical studies of this lake have been conducted as part of the Skeena River Salmon Investigation. Its area of 5.2 square miles (14.2 sq. km.) and mean depth of 24 feet (7.9 m.) provide a relatively small volume of water which is subject to considerable circulation from heavy winds sweeping in from the Pacific coast. Summer thermal stratification is varied and irregular, resulting in a proportionately large and unstable epilimnion. Except for a single bottom depression, oxygen concentrations remain at a high level throughout the year.

During four months of the year the lake is ice covered. Large snow and ice fields provide an excellent reservoir for stream flow.

Among the Skeena river lakes Lakelse is one of the most productive of sockeye salmon. Their survival does not appear to be limited directly by physical or chemical factors.

INTRODUCTION

During the five years, 1944 to 1948, physical and chemical studies were conducted on Lakelse lake, B.C., as part of the programme of salmon research on the Skeena river (Pritchard 1947). It was the express purpose of "The Skeena River Salmon Investigation" to examine the conditions for all species of salmon with special interest in the sockeye salmon, *Oncorhynchus nerka*. Primarily the inyestigation was designed to establish conservation measures.

The life cycle of the sockeye usually involves at least one year of lake residence. It is during this lacustrine stage that the greatest mortality befalls the young sockeye (Foerster 1938; Brett 1948). In recognition of the significance of lake studies in this investigation general surveys of the more important bodies of water in the Skeena river drainage were conducted. Lakelse lake was singled out for particular attention in an effort to elucidate the more limited findings in other areas by a critical study of one area.

While these data represent strictly a record and study of the physical structure of the lake, an effort has been made to demonstrate their relationship to sockeye survival, both young and old.

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

PHYSIOGRAPHY AND MORPHOMETRY

LOCATION AND GEOLOGY

Lakelse lake, 54° 30′ N., 128° 40′ W., is situated seventy miles directly east of the coastal city of Prince Rupert and about ten miles south of the Skeena river. To the west and east of the lake, mountains rise to 5,000 feet (1,500 m.), forming a basin two to seven miles wide which crosses the Skeena valley at Terrace and extends south to the Kitimat arm of the Pacific. A low flat-topped rise of 300 feet (92 m.) in the valley basin just south of the lake forms the division of drainage between the lake and the Kitimat arm. The geological structure and fossil remains indicate that the area was once continuous with the ocean, a possibility

FIGURE 1. Looking north on Lakelse lake from a central position.

which is readily conceived by studying the land elevations. The height of the lake itself is reported as 220 feet (67 m.), about 30 feet (9.2 m.) above the level of the Skeena river at the point of confluence with the Lakelse river.

The mountains of this area constitute a portion of the eastern margin of the Coast Range which was lifted up from pre-existing rocks during the Jurassic period by enormous upwellings of igneous magmas. These rocks in the form of multiple batholiths consist mainly of granite, diorite and quartz porphyry. The exposure of the basin itself is of more recent Pleistocene time and is mainly alluvium and glacial silt (Marshall 1926).

During the evolution of the Glacial age much of this whole coastal region was depressed 500 to 600 feet by an immense thickness of ice. With its recession

and the consequent uplifting of the land masses, probably some 20,000 years ago, the connection and drainage to the Kitimat arm was severed and dammed up to form the present lake (Hanson 1923).

Climate

The climate of the Lakelse lake area can be judged from the records taken at the meteorological station at Terrace, ten miles north of the lake, and from later records taken during the spring and summer months at the lake itself. These latter indicate a slightly greater precipitation and a somewhat more moderate

FIGURE 2. A view of the northern end of Lakelse lake from Mt. Layton, 2,000 feet.

temperature at the lake when compared with those from Terrace (May to September), although very similar weekly means exist. The body of water and the criss-crossing mountain ranges of the Lakelse and Skeena river valleys would account for such local differences.

The annual mean temperature for Terrace during the 33-year period from 1913 to 1945 (B.C. Climate 1945) was 44°F. (6.7°C.). The temperature rarely rises above 90°F. (38°C.) or falls below 0°F. (− 18°C.). Frequent heavy snowfalls and continued frost often prolong the winter well into April in the heavily

wooded country surrounding the lake. In this manner a considerable reservoir of water is retained in the form of snow and ice particularly on the mountain sides.

The spring is often a time of floods, gouging out river beds and cutting new courses, frequently leaving portions of the old waterways dry during a later period of reduced flow. This is a characteristic of a great deal of the Skeena river

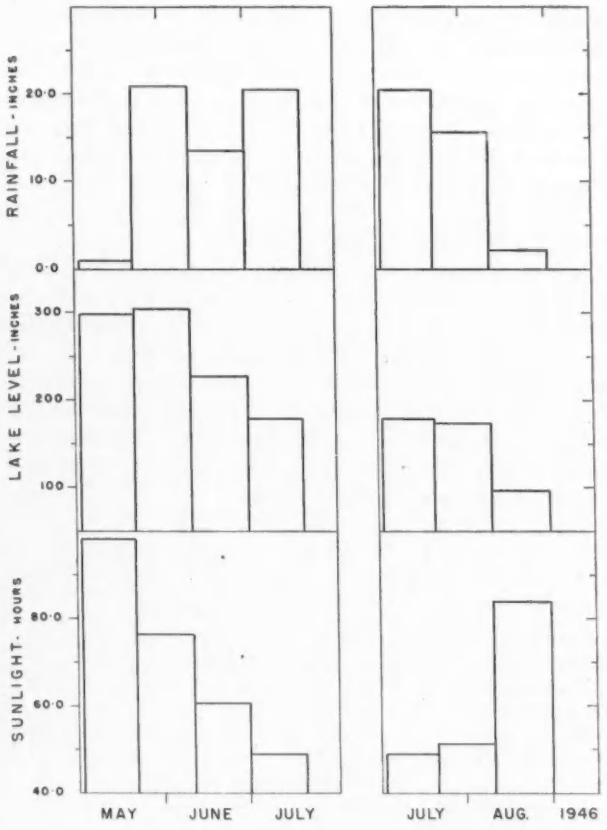


FIGURE 3. The relation between rainfall, hours of bright sunlight and lake level, plotted for 20-day intervals. The month of July has been repeated in a split graph to illustrate the difference in relation between early and late summer.

country and in both the spring and late fall constitutes a potential and unpredictable threat to the survival of salmon eggs from a given spawning.

The average precipitation for the same 33-year period is recorded (B.C. Climate 1945) as 46.85 inches (119.0 cm.). The months of May to August present the minimum period of rainfall, with an average of 2 inches (5.1 cm.) per month, following which there is a rapid increase of rain (and snow) to a peak in November

of 7.48 inches (19.0 cm.). The location of the lake is such that it is on the border between coastal weather conditions and the drier inland climate. As a result, it experiences considerable variation in weather, but can be considered as characterized by a moderate climate with fairly heavy rain and snowfall.

In the spring, lake and stream levels have been found to rise more in accordance with hours of sunlight (melting snow) than with precipitation, a relation which is gradually supplanted by the effect of precipitation alone with the progress of the seasons into the late summer months (fig. 3). By August and September when the adult sockeye are entering the streams, rainfall appears to be all important in maintaining the height of the rivers and consequent covering of the spawning beds. A reduction in the precipitation during these months in the Skeena river drainage appears to result in a possible reduction in the subsequent commercial catches of adult sockeye (cf. Hagman 1938).

Size, Shoreline and Bottom Configuration

The lake is approximately 5.4 miles in length along a N.E.-S.W. axis and varies from 0.7 to 1.5 miles in width, covering an area of 5.47 square miles, or 3,500 acres (14.2 km.^{2}). With a regular shoreline the shore development is not great, being just short of twice the minimum and equal to 1.83. It is characterized by many reed beds and very little rock. Fig. 4 illustrates the shape and the bottom configuration, the latter plotted to five metre intervals. The southern end is particularly shallow and reflects the general condition throughout, in which the mean depth is only 7.8 metres (24 feet). The areas and volumes for each 5 metre interval have been recorded in table I.

Table I. The morphometry of Lakelse lake.

Area	14.17 sq. km. (5.47 sq. mi.)
Maximum length	8.7 km. (5.4 mi.)
Maximum width	2.4 km. (1.5 mi.)
Maximum depth	32.2 m. (98 ft.)
Mean depth	7.9 m. (24 ft.)
Volume	108.0 × 10^6 cu. m. (141.3 × 10^6 cu. yd.)
Shore development	1.83
Elevation	72.2 m. (220 ft.)

Depth (m.)	Area (hectares)	% total surface area	Stratum (m.)	Volume (cu. m. × 10^6)	% total volume
0	1420	100.0	0-5	55.7	51.6
5	808	77.0	5-10	30.4	28.1
10	412	29.1	10-15	15.4	14.3
15	205	14.4	15-20	4.9	4.5
20	52	3.7	20-25	1.4	1.3
25	3	0.2	25-32.2	0.2	0.2
			Total	108.0	

The particular feature of Lakelse lake which defines so much of its nature is its mean depth. Young sockeye are considered to be limnetic rather than littoral in habit during the summer months (Ricker 1937b). Since the lake is so shallow

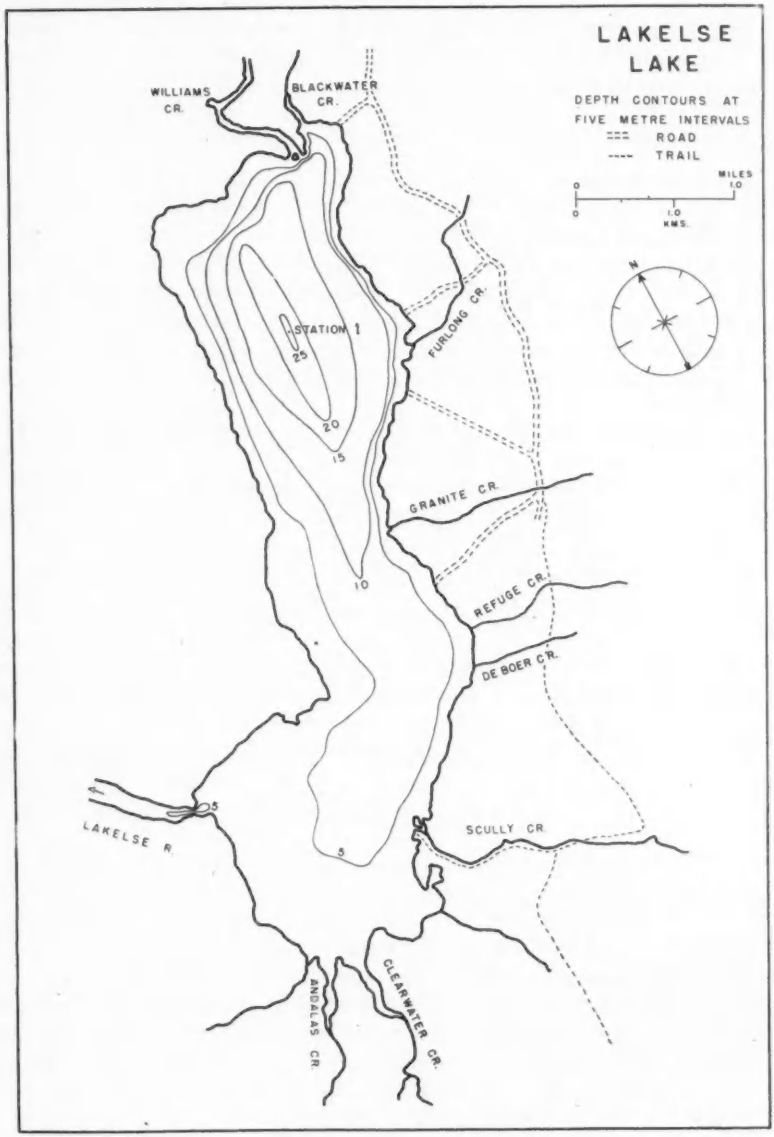


FIGURE 4. Map of Lakelse lake, British Columbia, showing bottom configuration.

the limnetic zone is correspondingly reduced and the average littoral temperatures rise as high as 20°C. (68°F.). This promotes growth of aquatic plants and animals resulting in a greater unit abundance of food, and a lake capable of supporting larger populations of young fish. Although restricting the inhabitable zone for young sockeye the net balance appears to be one of favourable conditions for their production. In the spring of 1948 it was estimated that between one to two million yearling sockeye migrated seaward.

Drainage and Water Level

The main stream drainage into the lake is that from Williams creek at the northern end, supplemented by the lesser streams of Granite and Scully creeks from the east, and Clearwater and Andalas creeks from the south. The outlet is via the Lakelse river in the southwest which flows almost directly northwest to the Skeena twelve miles distant. With the exception of the first mile, the Lakelse river has an even, rapid flow over coarse gravel and boulders. The mouth is narrow and deep, expanding into a wide, shallow arm with little noticeable current and preceding the more turbulent portion of the river downstream.

The general characteristics of these rivers have been outlined in table II:

Table II. Physical characteristics of the main streams entering Lakelse lake.

River	Summer temp.	pH		Clarity	At outlet		Bottom
		Red	Blue		Width	Depth	
Williams cr.	8-12°C.	7.3	7.1	Fairly turbid	1. 50′* 2. 80′ 3. 15′	4′	Gravel and sand
Blackwater cr.	17-20°C.	—	6.5	Fairly clear	60′	5′	Mud, silt and sand
Granite cr.	8-10°C.	7.2	7.0	Clear	25′	2′	Sand and gravel
De Boer cr.	9-11°C.	7.3	6.9	Clear	25′	1′	Sand and gravel
Scully cr.	8-10°C.	7.3	7.2	Clear	25′	3′	Sand and gravel
Clearwater cr.	10-13°C.	7.9	7.7	Clear	200′	2′	Sand and silt
Andalas cr.	7-10°C.	7.9	7.6	Turbid	200′	2′	Silt

*Three separate outlets.

For the past five years approximately 80% of the total adult sockeye run has entered Williams creek. While spawning they distribute themselves over its many suitable gravel beds for a distance of four to four and one-half miles (Pritchard and Cameron 1940). The major supply of water to the lake comes from this source which is mainly glacial and run-off in origin. Williams creek, in providing the spawning beds, constitutes one of the main physical features of importance in a salmon-producing body of water.

The fluctuations in volume discharge of Williams creek are reflected by fluctuations of water level within the lake. A continuous record of the lake water level provides a picture of the variations in river height. Peaks of high water usually occur in May following melting of snow under intense sunlight and in late October or early November as a result of heavy precipitation. The low levels occur between the end of August and the beginning of September, and just prior to ice formation (fig. 5).

The late summer minimum coincides with the major spawning period. If there is a drought at this time, a reduction in the amount of spawning beds may

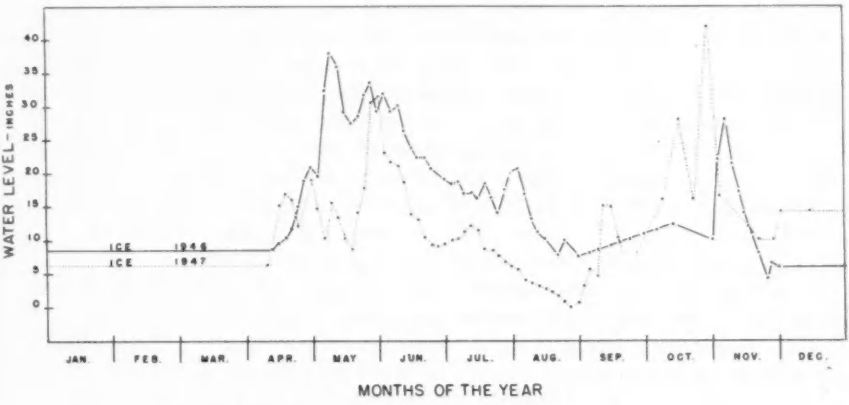


FIGURE 5. Annual variation in water level for 1946 and 1947 at Lakelse lake.

seriously reduce the efficiency of egg deposition. If abnormally high water exists many beds may be left exposed in a subsequent drop of level. It would appear that an average level somewhere near that of the final freeze-up level would be most suitable for successful production of young fish from a given seeding.

TEMPERATURE

PROCEDURE

The temperature variation has been recorded for Lakelse lake at a single station in the vicinity of the greatest depth, Station 1, during the months of July to September of 1944, April to September 1945, and at two week intervals throughout all months of the year from April, 1946, to May, 1948. Throughout this study a standardized Negretti-Zambra deep-sea reversing thermometer, graduated to fifths of a degree Centigrade, has been used (Kemmerer *et al.* 1923). In addition, a bathythermograph (Spilhaus 1937) was used in 1946 and operated for more extensive recordings in the early part of each season's work. Its use was directed toward determining the thermal structure of the whole lake rather

than of one column, thus testing the degree of representation afforded by the single station. Since the lake is characterized by one main depression the complications of thermal structure introduced by submerged depressions (Welch and Eggleton 1932) are not present.

SEASONAL CHANGES

1. *Spring Overturn:* With moderate climatic conditions in winter, the ice coverage is not excessive, amounting to a maximum of twenty-four inches of porous snow-ice intermingled with layers of hard ice. Toward the end of March in an average year, sunshine and warm winds constantly melt the surface and reduce the thickness. The break-up varies from abrupt upheaval to gradual displacement depending on the meteorological conditions which precede it. Once the ice has been reduced to a few inches and shows definite cracks and breaking points a heavy wind can be disruptive, the actual break-up being followed rapidly by the spring overturn and a rise in surface temperature.

For the years 1945 to 1947 the overturn has occurred within one day of April 4, while in 1948, which was an exceptional year, the overturn did not occur until April 24. The usual state is a break-up and overturn in the first week of April.

2. *Thermocline Formation and Period of Summer Stagnation:* The presence of a distinct thermocline* rarely becomes apparent in the first month of heat interchange but thermal stratification is present from the very first days and an upper circulation of water of different depths occurs to varying degrees in proportion to the intensity and duration of the wind. This is in direct contrast to the period leading up to ice formation in the fall of the year.

During the summer intense winds, sweeping up the open Kitimat Arm of the coast and through the Lakelse lake valley, often disrupt the thermal layering of the lake resulting in marked fluctuations in the presence, absence or position of one or more true thermoclines. The very deepest portion of the lake, however, remains at least partially out of circulation. Lakelse lake is consequently a poor example of the classical concept of a thermally stratified lake in midsummer. It might be described best as a lake which undergoes thermal stratification of varying degrees, sometimes exceeding 1°C. per metre, with an upper zone of water which tends to circulate as a separate body and which becomes more distinct as a maximum in water temperature is approached. This zone of water usually varies from 10 to 20 metres in depth and yet may be dispersed within a few hours by wind action.

The maximum surface and bottom temperatures for each year appear in table III. The average peak condition (1944-47) has come some time in the month of August when the whole lake became warmed to an extent which resulted in temperatures averaging 19.1 ± 0.9°C.† at the surface to 10.9 ± 1.4°C. at the bottom.

*Defined as equal to or greater than a change of 1°C. per metre (Welch 1935).

†Standard deviation used throughout.

TABLE III. Surface and bottom temperatures at the time of maximum thermal state, Station I, Lakelse lake.

Year	Date	Surface	Bottom
1944	Aug. 8	19.4°C.	13.0°C.
1945	" 8	20.2	9.2
1946	" 29	18.8	10.3
1947	" 15	17.8	11.1
Average	Aug. 15	19.1 ± 0.9°C.	10.9 ± 1.4°C.

3. *Fall Overturn:* By September all strata of the lake with the exception of the greatest depths have commenced to cool. A month later the whole lake shows a declining temperature, the upper strata losing heat more rapidly than the lower strata, resulting in final uniformity of temperatures throughout. In 1946 this occurred by approximately October 31 with a uniform temperature of 7.0°C., while in 1947 a consistent and almost parallel decline of temperature for all strata commenced in early October and finally merged just prior to reaching 4°C. This marks the start of the overturn which continues until a new stratification in reverse occurs as cooling below 4°C. proceeds. By the end of November ice has usually sealed the lake from further wind-produced currents. Table IV contains a summary of the data for duration of overturn and commencement of surface freezing.

TABLE IV. Fall overturn and winter data, Lakelse lake.

	Fall overturn			Winter stagnation		
Year	Commenced	Temp.	Date 4°C. reached	Date of freeze-up	Min. bottom temp.	Date of break-up
1946-47	Oct. 31	7.0°C.	Nov. 8	Nov. 24	3.7°C.	April 15
1947-48	Oct. 15	11.0°C.	Dec. 8	Dec. 20	3.2°C.	April 23

4. *Winter Stagnation:* The sharp drop in temperature ceases for the lake as a whole when a temperature of 4°C. has been reached. Only the surface waters continue to cool giving rise to the usual inverse thermal stratification. The extent of the upper stratification at first exceeds 1°C. per metre of depth. As further cooling takes place and the ice surface is established this gradient is reduced. The temperatures at each depth progressively drop below 4°C. until the more stable bottom temperatures are involved. A reduction of temperature at 80 feet

has been apparent in both 1947 and 1948. A record of the temperature variation at 20 foot intervals of depth from April 1946 to April 1948 has been depicted in fig. 6.

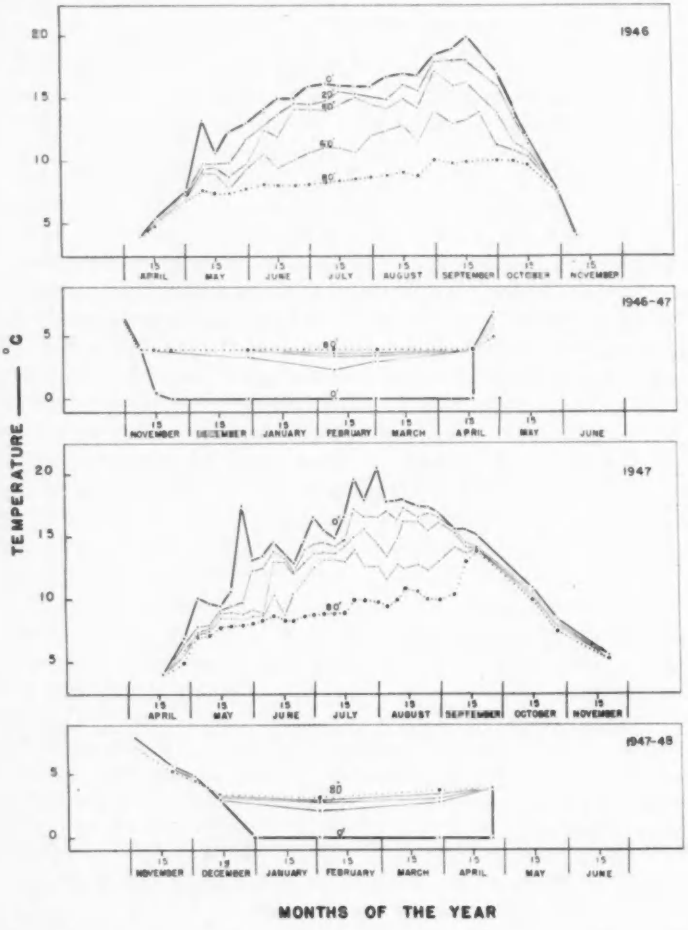


FIGURE 6. Temperature variation at depths of 20-foot intervals, Station 1, from April, 1946 to April, 1948. (The data used in plotting these graphs are available on request from the Pacific Biological Station, Nanaimo, B.C.)

With the elimination of wind effect by an ice blanket the thermal changes conform to the state described for most ice-covered lakes in a temperate climate (Welch 1935; Greenbank 1945). The term "stagnation" however is somewhat misleading here in that it would imply a marked stability for the water of greatest

depth and the absence of any circulation within its bounds. That there may be some exchange as a result of river water displacement would appear possible through inference from the high oxygen content recorded throughout the winters of 1946-47 and 1947-48.

Yoshimura (1935) sub-divided the stagnation periods of fresh water lakes into eight distinguishable phases in an attempt to elucidate further the thermal evolution within a lake. Since temperature changes are dynamic in nature, division into different categories is in part arbitrary, relying on the importance attached to aspects of the study by some particular investigator. In the case of Lakelse lake, being in a north temperate zone, the two physical features which result in major differences in thermal stratification are the reduction in temperature through 4°C. and the freezing of the surface. Further subdivision of the annual temperature cycle is not particularly appropriate. The volume of water over 15 metres in depth is only 6% of the total volume. If a portion of this did become stagnant during the summer, it could hardly be considered representative of the whole lake.

Heat Budgets

Attempts to set a measure of the effect of solar energy in terms of heat units and provide a means of comparing different lakes were advanced through the work of Forel (1901) and Halbfass (1905, 1913), and later developed by Birge (1915) and Birge and Juday (1914, 1921, 1929) in terms of an annual heat budget. The significance of heat budgets has been questioned by each of the above investigators and the conclusions are conflicting. In particular Birge and Juday (1914) have defended and developed its use as a comparative index. Birge (1915) states, "No doubt more complete study will show that the heat budgets of the several lakes are influenced by elevation, surroundings, size of affluents and effluents, climate, cloudiness, latitude, and other conditions as well; but at present a direct and unmistakable effect of any one of these conditions can be pointed out only in a very few cases, and it is best to consider the budgets in gross and without too much attention to particular circumstances" (p. 167). "It allows us to determine the influence of such factors as the area and depth on the amount of heat taken in by them" (p. 176). Ricker (1937a) in his study of Cultus lake, B.C., comments that "The small heat budget of Cultus lake is a direct reflection of the generally equable climate of the region" (p. 376)

Whatever the significance may be, it is interesting to compare the annual heat budgets of Lakelse lake with those from two other sockeye producing lakes on the Pacific coast, Karluk lake, Alaska (Juday *et al.* 1932) and Cultus lake, B.C. (Ricker 1937b). The immensity of the sockeye populations (Gilbert and Rich 1927) which migrate both into (adults) and out of (young migrants) Karluk lake necessitates a colossal conversion and production of food. Cultus lake, classified as oligotrophic by Ricker (1937a), is nearly one-sixth the area of Karluk lake, but only produces by comparison about one one-hundredth as many sockeye (Foerster 1938). Lakelse, in proportion to its area, has a smaller run of adult sockeye than Cultus lake (it is over twice the area of Cultus yet has just half the

number of sockeye). In table V the heat budgets in gm.-calories/cm² from 1946 to 1948 are shown. The average of 13,110 for Lakelse is considerably lower than that of 24,300 for Cultus and much more so for Karluk at 33,500. Lakelse lake appears to have one of the lowest heat budgets on record.

TABLE V. Heat budgets and summer heat incomes for Lakelse lake in gm.-calories/cm².

Year	Heat budget	Summer heat income
1946	12,450	11,150
1947	13,350	12,050
1948	13,540	12,160
Average	13,110	11,190

DISCUSSION

As stated previously the fry and yearlings appear to frequent the limnetic zone, avoiding the littoral region, perhaps because of a temperature intolerance or purely from thermal preference. The latter view is supported by the work of Donaldson and Foster (1941) who state, "From the experimental evidence and field observations, it would appear that the young sockeye salmon are very selective in their choice of water of uniform temperature, choosing that water near the thermocline in preference to warmer surface water or colder waters in the depths of the lakes". They also conclude that "the temperature of the surface layer of water in the lake (Skaha lake, B.C.) was too high for optimum growth, survival, and efficient utilization of food". Ricker (1937b) has stated that "the summer feeding of fingerlings appears to be chiefly confined to the region between 5 and 15 metres depth, which includes the thermocline and adjacent narrow strips of the epilimnion and hypolimnion. Foraging is limited upward by scarcity of food, and downward by poor illumination or low temperature". The latter conclusion is one made more by inference than direct observation. The same can be said for Lakelse lake, for, with the exception of one single observation, fingerlings have not been caught or observed during the summer in the littoral zone which is the only area where suitable fishing gear has been employed.

Temperature as a direct lethal factor (cf. Fry 1947) and temperature as an indirect factor promoting death through disease have different operative levels. The effect of the myxobacterium *Chrondrococcus columnaris* as a threat to the survival of salmon under given temperatures has been demonstrated by Fish (1948) and Fish and Rucker (1943). The former states (1948) that, "Below 60°F., columnaris disease is of little consequence, but between 60° and 70°F., *C. columnaris* invades the inevitable cuts and abrasions on fish, quickly establishing a secondary infection that may prove lethal. Above 70°F., *C. columnaris* becomes a pathogen in its own right and needs no mechanical injuries to open a door through the protecting mucous coating of the skin and gills".

No significant examples of either direct or indirect effects of temperature causing high mortality in sockeye have been observed at Lakelse lake. Its fundamental effect appears to be one of promoting sockeye growth and activity while limiting the scope of foraging mainly to the limnetic and thermocline zones.

TRANSPARENCY

Procedure

As a measure of transparency a standard Secchi disc was used at the time of making bi-weekly recordings in the centre of the lake at Station 1. By observing the usual methods of operating this instrument, and recording the accompanying weather conditions, a broad classification of the Skeena river lakes has been accomplished.

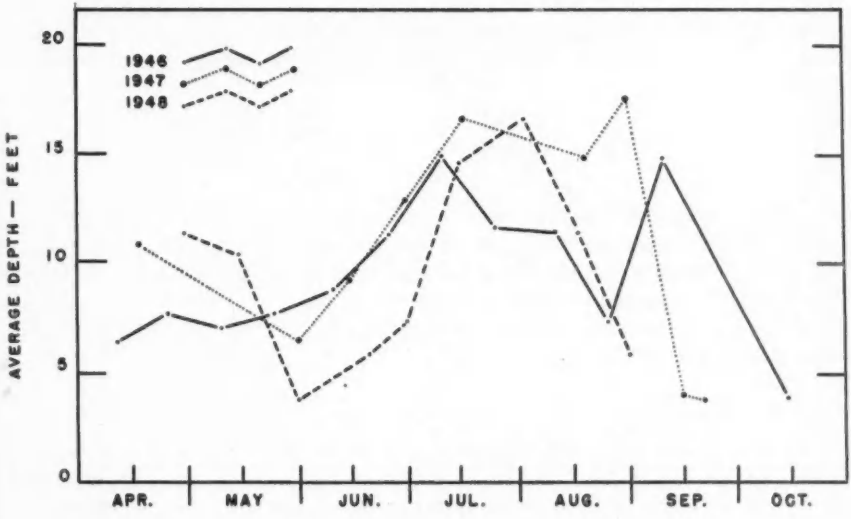


Figure 7. Secchi disc recordings at Station 1, Lakelse lake, 1946-1948.

Variation and Average Transparency

A peak in transparency has repeated itself in July and again in September in every summer of continuous observation on Lakelse lake, while the end of May and the middle of October have been characterized by heights of turbidity (fig. 7). These latter two coincide with periods of flood and may be compared with the graph of water level fluctuation, fig. 5. The lake cannot be considered as a heavily silted lake for its average transparency from the records of 1945-48 is 10.0 feet (approximately 3 m.) and a record of 17.5 feet (5.3 m.) exists for August 30, 1947.

Although the main stream discharge into the lake stems from mountain sides of the surrounding terrain and results in a partial silting of the waters, the obstruction to light penetration on an average day must come as much from

microscopic organisms and organic matter as from inorganic suspended particles. The temperature relations are probably as responsible for this phenomenon as any other factor. In the summer season, the temperature of the epilimnion is almost invariably higher than the temperature of the river waters, thus the silt tends to be carried down to the hypolimnion zone, leaving the upper waters comparatively clear. The reverse of this has been found to be true for other colder lakes in the same drainage.

The lower limits of sight and of food abundance are very reduced in certain of the Skeena river lakes, considerably more so than in Lakelse. A preliminary survey would indicate that the suitability of a lake for sockeye production is closely linked with transparency. This may be a direct result of limitation of phyto-plankton by a reduction in light penetration. However, the presence of heavy silt in a lake reflects an even heavier suspension in the inflowing rivers, and such features reduce the area of good spawning beds. It is probable that the effects of silt in both lake and stream combine to limit the productive capacity for sockeye.

DISSOLVED OXYGEN

Procedure

With a view to recording oxygen concentrations, samples of the water at various levels, always including the surface, usually the thermocline region, and one sample within a few feet of the bottom, were taken with a Kemmerer water bottle as routine procedure while determining temperature gradients at Station 1. These samples were analysed for total oxygen by the Winkler method of determining dissolved gas (Theriault 1931).

Seasonal Changes

The variation in oxygen content of any lake is mainly a product of organic decay, animal metabolism and physical dynamics. During the spring and fall overturns no distinct deficit can occur for purely physical reasons. The periods of major concern, biologically, are during the summer and winter stagnation periods (Birge and Juday 1911; Greenbank 1945). The variation in oxygen saturation at depths of 0, 40 and 80 feet have been plotted in fig. 8 which is a typical example of the conditions at Lakelse lake for that portion of the year. No record has been taken of an oxygen saturation below 45% (5.2 p.p.m., 9.7°C., 80 ft., September 14, 1945) and since this degree of reduction is restricted to the hypolimnion very little diminution of the total oxygen content occurs. It might be expected that winter stagnation would result in a more pronounced deficit, but all attempts to measure this have indicated a consistently high level of oxygen saturation, e.g. March 1, 1947, 10.1 p.p.m. at 80 feet, 3.8°C., 76% saturation, 13 inches of ice.

It will be noted that at times the surface oxygen rises above 100% saturation, although only slightly. Each of these occasions was marked by rising surface temperatures and calm water conditions. Since the solubility of oxygen in water

decreases with rising temperature the above facts would readily explain the observed phenomena.

Undoubtedly an active wind circulation and unstable thermocline during the summer, with a submarine flow pattern during the winter, play important yet undefined roles in the oxygen saturation at all levels in the lake.

Discussion

Gardner and Leetham (1914) have shown experimentally that the amount of oxygen required by fish varies with the temperature of the water. Asphyxial points for brown trout vary from 1.13 p.p.m. at 43.5°F. (6.4°C.) to 3.4 p.p.m. at 77.0°F. (25°C.). This is also essentially true for brook trout and rainbow trout

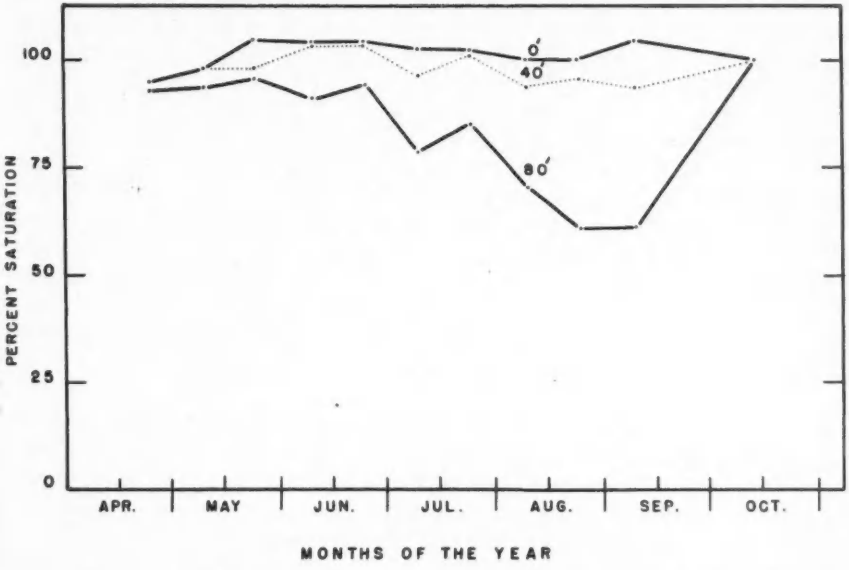


Figure 8. Variation in oxygen saturation at 0, 40, and 80 feet, Station 1, Lakelse lake, 1946.

(Gutsell 1929). Townsend (Townsend and Earnest 1940; Townsend and Cheyne 1944) has reviewed various minimum oxygen tolerances of salmonoid fishes and points out that these minima differ by nearly eight times the lowest value observed and that the highest is that reported by Gardner and Leetham, cited above.

With this information and taking into account that no consideration has been given to many possible complicating factors such as increased carbon dioxide (Creaser 1930; Shaw 1946), a general statement that, unless oxygen concentrations of 30% (i.e. about 3.5 p.p.m. or 2.5 cc. per liter at 10°C.), or less, are discovered in some region of the water body of a lake, then it is most likely that oxygen is not a limiting factor to either the distribution or existence of any species of fish within the water system. This has been shown to be essentially true for

sockeye by Chapman (1939) who found that distress from low oxygen could be demonstrated experimentally at or below 3.5 p.p.m. with accompanying high temperatures (up to 19.2°C.) and with an above normal concentration of carbon dioxide (up to 2.3 p.p m.).

The immediate application of this conclusion for Lakelse lake would be that sockeye are in no way limited by oxygen deficits and that, in any consideration of their high lacustrine mortality the problem of oxygen deficiency, at least for the present, can be set aside.

HYDROGEN ION CONCENTRATION OR pH

Procedure

It is routine procedure in lake studies to make systematic recordings of the extent of acidity or alkalinity of the water, particularly in the deeper sections where experience has taught that the extremes are most likely to be met. The significance of the pH, especially when no determinations of carbon dioxide concentrations are made, may be useful as an index of certain environmental conditions (Rawson 1939). At Station 1, pH recordings were made at various levels including the surface and bottom (not closer than 2 feet from the actual bottom) using a Taylor pH Slide Comparator Model T-O, with a Bromthymol Blue Slide (pH 6.0-7.6) and a Cresol Red Slide (pH 7.2-8.8), manufactured by W. A. Taylor & Co., Baltimore, Md. Wherever possible, duplicate readings were made on the same samples of water with the two slides. From the discrepancies recorded and further checks on the reliability of such colorimetric field determinations, it became apparent that many inaccuracies may and do occur with this system.

Results

Using the bromthymol blue slide the average summer readings with their standard deviations were:

$$\text{Average surface pH} = 7.1 \pm 0.2$$
$$\text{Average bottom pH} = 6.6 \pm 0.2$$
$$\text{Difference} = 0.5 \pm 0.28$$

The difference is slight. Throughout the epilimnion the pH was quite constant, usually with a slight drop through the zone of thermal stratification, and a somewhat greater drop in the hypolimnion. Being close to the neutral point (pH 7.0) and exhibiting so little divergence from this state it might be inferred that water circulation and gaseous exchange are quite unimpeded, that no excess of CO_2 accumulates, and that the products of bottom decay are well dispersed. In addition the pH of the inflowing rivers is 7.0 ± 0.7 (table III) from which it can be concluded that the surrounding area of drainage is not influenced to any excess by acidic or alkaline deposits.

Discussion

Lakelse lake typifies a body of water with very little variation in pH, and which is only slightly acidic. Investigations to date indicate that even such sensitive fish as trout can withstand wide variations in pH (Needham 1940) and

that it is of little importance in affecting their distribution. Creaser (1930) states that the "range of tolerance by brook trout varies at least from 4.1 to 9.5, and furthermore the hydrogen-ion concentration throughout this range does not seem to shift the voluntary toleration limits either of temperature or of dissolved oxygen content". Even sudden changes of fair magnitude, 2.0 above and below the neutral point, can be tolerated by certain species of Centrarchidæ (Wiebe 1931). Thus, with the exception of possible microstratification of the bottom, further consideration of pH in Lakelse lake would seem unwarranted with respect to its influence on, or limitation of, animal life within the lake.

CLASSIFICATION

Lakes have been classified on various bases, mainly from separate and collective considerations of mean depth, oxygen content, temperature, bound carbon-dioxide, dissolved nutritive material, plankton communities and bottom fauna. Welch (1935, 1941) and Rawson (1939) have reviewed and criticized these classifications pointing out some of their useful aspects and short-comings. Biologically, if they constitute an index of productivity then their usefulness and application should come in large measure from this aspect.

In such a lake as Lakelse the epilimnion constitutes nearly 90% of the water volume by mid-summer and might well be expected to have an amount of oxygen far exceeding that of the hypolimnion, regardless of any discrepancy in the amount of oxygen per unit volume in the two strata. From oxygen relations alone it becomes an eutrophic lake. Its mean depth is such as to support this classification, yet its relative production of sockeye when compared with the oligotrophic Cultus lake is less and its plankton population is not particularly abundant.

Unquestionably it may be placed among the temperate lakes, order 2, (Whipple 1927), i.e., surface temperatures vary above and below 4°C.; temperature of bottom water varies only slightly from 4°C.; it has two circulation periods.

During the course of study on the Skeena river it was convenient to classify the different lakes into two distinct groups, adding a third class called "Intermediate" merely to place those which actually were intermediate in a separate group. The two major categories were: (1)deep, cold bodies of water distinctly opaque and grey from glacial silt, and (2) rather shallow bodies of water, clear, of moderate temperature, and abundant in plant life. Lakelse lake is an example of the second type and, as such, is basically eutrophic.

SUMMARY

1. Lakelse lake, in the Skeena river drainage, is a small (3500 acres), shallow (24 feet mean depth) lake, situated on the eastern margin of the Coast Range mountains. The district is of moderate climate with relatively heavy snowfall providing glacial sources for stream drainage.

2. Heavy winds tend to keep the greater part of the lake in circulation during the summer, resulting in an unstable thermal stratification and bottom

temperatures which rise to 10 and 11°C. Ice coverage usually lasts from December to early April.

3. Although the transparency varies considerably it averages 10.0 feet (Secchi's disc).

4. The dissolved oxygen remains high all year round and is only reduced in a very restricted hypolimnial zone. The minimum on record was 45% (5.2 p.p.m., 9.7°C.).

5. The pH was very stable throughout.

6. From its physical features the lake may be classified as eutrophic and temperate (second order).

7. A discussion of the application of these findings to the limitation of sockeye production has been included.

ACKNOWLEDGEMENTS

As noted in the introduction, this study has been conducted as a part of the Skeena River Salmon Investigation of the Pacific Biological Station. Dr. A. L. Pritchard in charge of the investigation, and in co-operation with Dr. R. E. Foerster, has directed the operations. His encouragement, assistance and criticism have furthered the ends immeasurably.

During the period of five years devoted to this research various assistants have maintained records and conducted portions of the survey. In particular Mr. D. F. Alderdice and Mr. J. A. McConnell have contributed to the study. I am also indebted to Mr. A. C. Johnson and Mr. M. P. Shepard for their contributions.

REFERENCES

BIRGE, E. A. The heat budgets of American and European lakes. *Trans. Wisc. Acad. Sci., Arts and Lett.*, **28**, 166-213, 1915.

BIRGE, E. A. AND C. JUDAY. The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. *Bull. Wisc. Geol. Nat. Hist. Surv.*, **22**, Sci. Ser. 7, 1-259, 1911.

A limnological study of the Finger lakes of New York. *Bull. U.S. Bur. Fish.*, **32**, 527-609, Doc. No. 791, 1914.

Further limnological observations on the Finger lakes of New York. *Bull. U.S. Bur. Fish.*, **37**, 211-252, Doc. No. 905, 1921.

Transmission of solar radiation by the waters of inland lakes. *Trans. Wisc. Acad. Sci., Arts and Lett.*, **25**, 285-335, 1929.

BREHM, V. AND F. RUTTNER. Die Biocönosen der Lunzer Gewässer. *Int. Rev. Hydrobiol.*, **16**, 281-391, 1926.

BRETT, J. R. The design and operation of a trap for the capture of migrating young sockeye salmon. *Trans. Am. Fish. Soc.*, **75** (1945); 97-104, 1948.

CHAPMAN, W. M. Effects of a decreased oxygen supply on sockeye and chinook salmon. *Trans. Am. Fish. Soc.*, **69**, 197-204, 1939.

CREASER, C. W. Relative importance of hydrogen ion concentration, temperature, dissolved oxygen, and carbon dioxide tension on habitat selection by brook-trout. *Ecology*, **11**, 246-262, 1930.

DONALDSON, L. R. AND F. J. FOSTER. Experimental study of the effect of various water temperatures on the growth, food utilization, and mortality rates of fingerling sockeye salmon. *Trans. Am. Fish. Soc.*, **70,** 339-346, 1941.

FISH, F. F. The return of blueback salmon to the Columbia river. *Sci. Monthly,* **66,**(4), 283-291, 1948.

FISH, F. F. AND R. R. RUCKER. Columnaris as a disease of cold-water fishes. *Trans. Am. Fish. Soc.*, **73,** 32-36, 1943.

FOERSTER, R. E. An investigation of the relative efficiencies of natural and artificial propagation of sockeye salmon (*Oncorhynchus nerka*) at Cultus lake, British Columbia. *J. Fish. Res. Bd. Can.* **4** (3), 151-161, 1938.

FOREL, F. A. Etude thermique des Lacs du Nord de l'Europe. *Arch. Sci. Phys. et Nat.*, **12** (7), Geneva, 1901.

FRY, F. E. J. Effects of the environment on animal activity. *Pub. Ont. Fish. Res. Lab.*, **68,** 1-62, 1947.

GARDNER, J. A. AND C. LEETHAM. On the respiratory exchange in fresh-water fish. Part I. On brown trout. *Biochem.* **8,** 374-390, 1914.

GILBERT, C. H. AND W. H. RICH. Investigations concerning the red-salmon runs to the Karluk river, Alaska. *Bull. Bur. Fish.*, **43** (2), 1-69, 1927.

GREENBANK, J. Limnological conditions in ice-covered lakes, especially as related to winter-kill of fish. *Ecol. Monog.*, **15,** 343-392, 1945.

GUTSELL, J. S. Influence of certain water conditions, especially dissolved gases, on trout. *Ecology*, **10** (1), 77-96, 1929.

HAGMAN, N. The variations in the catch of salmon and the water levels of the rivers. *Ann. Zool. Soc. Zool.-bot. fenn. Vanamo, Helsinki,* **5** (6), 1-43, 1938.

HALBFASS, W. Die Thermik der Binnen-seen und das Klima. *Petermanns Mitt.*, **51,** 219-233, 1905.

Einfluss der geographischen Lage auf die Wärmeverhältnisse von Seen. *Petermanns Mitt.*, **59** (2), 312, 1913.

HANSON, G. Reconnaissance between Skeena river and Stewart, B.C. Geol. Surv. Sum. Rep., Part A, 1923.

JUDAY, C., W. H. RICH, G. I. KEMMERER AND A. MANN. Limnological studies of Karluk lake, Alaska, 1926-1930. *Bull. U.S. Bur. Fish.*, **12,** 407-436, 1932.

KEMMERER, G. I., J. B. BARARD AND W. R. BOORMAN. Northwestern lakes of the United States. Biological and chemical studies with reference to possibilities in production of fish. *Bull. U.S. Bur. Fish.*, **39,** 51-140, 1923.

MARSHALL, J. R. Lakelse lake map-area, Coast District, B.C. Geol. Sur. Sum. Rep., Part A: 35-45, 1926.

NEEDHAM, P. R. Trout Streams. 1-233, Comstock Pub. Co. Inc., Ithaca, N.Y., 1940.

PRITCHARD, A. L. Fish cultural problems involved in the conservation of anadromous fish, with particular reference to Skeena river salmon. *Can. Fish. Culturist,* **1** (2), 8-13, 1947.

PRITCHARD, A. L. AND W. M. CAMERON. Observations on the sockeye salmon run at Lakelse lake (Skeena river) in the year 1939. *Prog. Rep. Pac. Biol. Stn.*, **43,** 14-16, 1940.

RAWSON, D. S. Some physical and chemical factors in the metabolism of lakes. *Am. Ass. Adv. Sci.*, **10,** 9-26, 1939.

RICKER, W. E. Physical and chemical characteristics of Cultus lake, British Columbia. *J. Biol. Bd. Can.*, **3** (4), 363-402, 1937a.

The food and food supply of sockeye salmon (*Oncorhynchus nerka* Walbaum) in Cultus lake, British Columbia. *J. Biol. Bd. Can.*, **3** (5), 450-468, 1937b.

SHAW, P. S. Oxygen consumption of trout and salmon. *Calif. Fish and Game,* **32** (1), 3-12, 1946.

SPILHAUS, A. F. A bathythermograph. *Sears Found. Jour. Mar. Res.*, **1** (1), New Haven, 1937.

THERIAULT, E. J. Detailed instructions for the performance of the dissolved oxygen and biochemical oxygen demand tests. U.S. Treas. Dept., Public Health Service, Suppl. No. 90 to the Public Health Reports, 1931.

TOWNSEND, L. D. AND H. CHEYNE. The influence of hydrogen ion concentration on the minimum dissolved oxygen toleration of the silver salmon, (*Oncorhynchus kisutch* (Walbaum)). *Ecology*, **25** (4), 461-466, 1944.

TOWNSEND, L. D. AND D. EARNEST. The effects of low oxygen and other extreme conditions on salmonoid fish. *Proc. Sixth Pac. Sci. Cong., Oceanog. and Marine Biol.*, **3**, 345-351, 1940.

WELCH, P. S. Limnology. 1-471. McGraw-Hill Book Co., New York, N.Y., 1935.

Dissolved oxygen in relation to lake types. Symp. on Hydrob., Univ. Wisconsin Press: 60-90, 1941.

WHIPPLE, G. C. The microscopy of drinking water. 1-409. John Wiley and Sons Inc., 4th ed., 1927.

WIEBE, A. H. Notes on the exposure of several species of fish to sudden changes in the hydrogen-ion concentration of the water and to an atmosphere of pure oxygen. *Trans. Am. Fish. Soc.*, **61**, 216-222, 1931.

YOSHIMURA, S. A subdivision of the stagnation periods of fresh-water lakes. *Archiv. Hydrobiol.*, **28**, 236-239, 1935.

Lakelse Lake Sockeye Survival

By J. R. Brett and J. A. McConnell
Pacific Biological Station, Nanaimo, B.C.

(*Received for publication November 30, 1949*)

ABSTRACT

The number of young sockeye migrating seaward from Lakelse lake, B.C., was 557,000 (1946) and 373,000 (1947). From seeding estimates in the parent years per cent survivals of 1.1 and 0.4 have been calculated.

Stomach analyses of 623 squawfish caught by gill nets in the late spring and summer months (1944-1947) show an average fish content of 83% by volume. Of this total fish content sockeye constitute 31%. The heavy losses in young sockeye could be attributed to the feeding of predacious fish and variation in sockeye survival may be a result of fluctuations in the abundance of "buffer" species.

INTRODUCTION

Sockeye (*Oncorhynchus nerka*), like most salmon, are anadromous in habit. Their life begins and ends in fresh water with an intermediate stage in salt water. They spawn in streams during the late summer and early fall, the eggs hatching in the gravel by January or February. Since spawning is mainly in streams above lakes, or immediately below, the young alevins after emerging in early spring are free to migrate directly downstream (or upstream) into the adjacent lake. Within the lake they remain to develop from fry into fingerlings. Seaward migration often takes place in the following spring but may not occur until one or even two years later. Various factors which may have a bearing on sockeye survival from egg to returning adult have been considered by Pritchard (1947) who presents a general picture of the problems of salmon conservation. Neave (1948) has pointed out the significance which may be attributed to the extent of egg production in four of the five species of *Oncorhynchus*, demonstrating an apparent direct relation between the known hazards of survival and the average number of eggs per female.

At Cultus Lake, B.C. (Foerster 1938a), and at Karluk lake, Alaska (Barnaby 1944), two major studies of sockeye propagation have been conducted. More recently a somewhat similar project has been undertaken at Lakelse lake, B.C. That the major losses occur in the fresh water stages has been conclusively demonstrated in each case.

The following comprises the history of investigation at Lakelse lake for the years 1946 to 1948, together with a consideration of possible controlling factors in the survival of sockeye.

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

Enumeration of Sockeye Migrants

The difficulties of successful capture and enumeration of the complete annual migration of many thousands of young fish present perplexing problems in any propagation study. The method employed at Lakelse lake has been described in an earlier paper (Brett 1948). In brief, the young migrating sockeye were directed into a central trap by netting leads which extended to either bank of the outlet river. A uniformly shallow but wide section of the river was chosen for the site of this structure. The results from three years of operation are outlined here in chronological order.

Figure 1. Dipping sockeye migrants for weighing in water.

During *1946* the migrants were weighed in water (fig. 1) and a count made of a weighed sample, equivalent to about 25% of the total run. The final sum of the daily calculations equalled 557,000. A randomly chosen sample of 50 migrants all proved to be in their second year and subsequent age determinations for later years have confirmed the observation that the majority migrate in their second year. Only by selecting larger specimens, quite obvious among schools of thousands of yearlings, were migrants over two years old obtained. In the parent year (1944) the total adult sockeye salmon run to Lakelse lake was estimated by use of a tagging ratio at 25,000 (Pritchard and Brett 1945). By applying to this figure the sex ratio recorded from counts made on the spawning grounds

(54% females) and using an average egg count of 3,888 per female obtained on Williams creek, Lakelse lake, (Pritchard and Cameron 1940) the survival from egg deposition to migrant was calculated as approximately *1.1%*.

The final aggregate of migrants in *1947*, also weighed in water, was 373,000. Since this smaller run was the product of an above average parent run of approximately 50,000 (estimated by tagging ratio method), the percentage survival

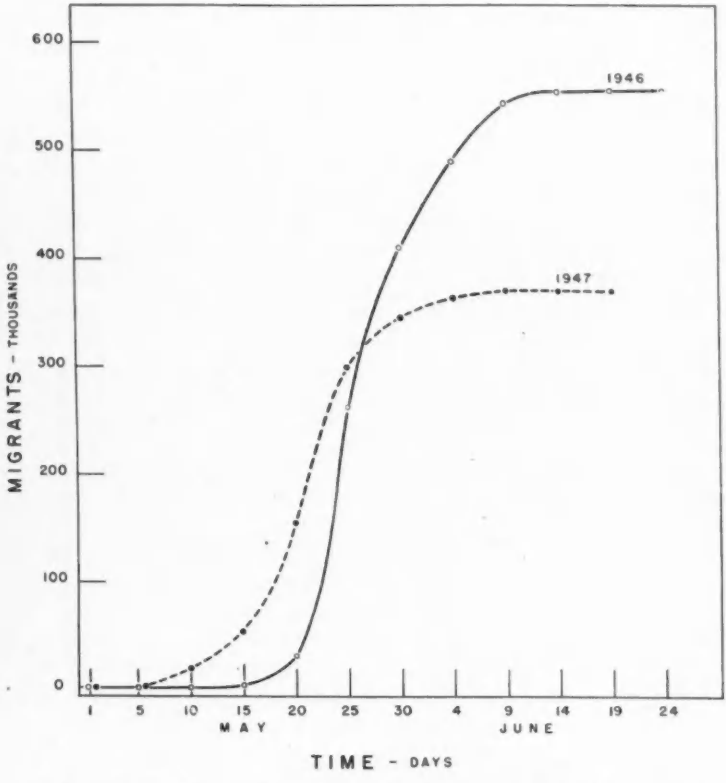


FIGURE 2. Migration of young sockeye down the Lakelse river in five day cumulative totals.

was only *0.4%*. This low survival has been reflected in a considerably below-average run of mature sockeye to Lakelse lake in 1949. The cumulative totals for the migrations in 1946 and 1947 have been plotted in fig. 2. While the period of migration in both cases extends from about May 1 to June 15, over 80% of the total enumeration has been made during a two week period in the middle of the run.

The installation of a new trap and fence across the Lakelse river in *1948* provided greater facility for capturing the migrants. Unfortunately at the very

peak of this migration exceptionally heavy floods swept a portion of the fence away and reconstruction could not be completed in time to give additional data of any value. By comparing the daily rate of increase in the first portion of the 1948 migration with that from the previous two years a run of 1,500,000 ± 300,000 was calculated. This figure would result from a survival rate of between 1% and 2%.

Again, it can be concluded that a very high mortality occurs during the fresh water stages of development, reducing the population to about 1/100 of the original.

Progress of Mortality in Young Sockeye

Egg to Fry (within the stream). No direct study* has been made of the mortality in sockeye eggs from deposition in the gravel to free swimming fry entering the lake. A method of liberating fry at different stages of development was used at Cultus lake, B.C., to calculate the mortality from lake residence alone. The survival recorded by Foerster (1938b) at the early fry stage was consequently

Table I. The per cent mortality in egg and alevin samples from seven sockeye redds.

Redd No.	Date sampled	Total sample	% Dead				% Live Alevin
			Infertile	Pre-eyed	Eyed	Alevin	
Williams creek no. 1	Feb. 2	194	0.0	3.1	8.2	0.0	88.7
" " " 2	" "	52	0.0	5.8	44.2	0.0	50.0
" " " 3	" "	56	0.0	0.0	0.0	5.4	94.6
Scully creek no. 1	Jan. 31	223	0.0	0.5	0.0	0.5	99.1
" " " 2	" "	107	4.7	5.6	27.2	1.9	62.6
" " " 3	" "	10	?	100.0	0.0	0.0	0.0
" " " 4	Mar. 25	625	0.0	0.0	9.4	0.2	90.4
Average of total		1,267	0.4	2.1	9.9	0.6	87.2
Estimated age (days)			0	15	35	150	150 +

an indirect calculation. The only opportunity to check this stage for losses at Lakelse lake was afforded by investigating seven marked redds following hatching in late January and prior to emerging in March of 1948 (table I). In this instance, after carefully collecting every bit of evidence from the excavated redds, an average survival of 87% was obtained. Of the dead eggs collected a good many were infected with the fungus *Saprolegnia*. Whether this was a primary or

*At present two investigations of mortality at this stage are being conducted by the Fisheries Research Board of Canada at Lakelse lake and Port John, B.C.

secondary infection was impossible to determine. Given that some dead eggs in a redd became infected with *Saprolegnia*, its growth might envelop others, finally killing these and providing a further source of nutrition for continued growth. Thus, the whole redd might be destroyed. The majority of the dead eggs were in the "eyed" stage. Very few were found to be infertile, an observation which adds confirmation to the figure for efficiency of fertilization for sockeye reported by Cameron (1940). This latter investigation, made on ten redds in Williams creek, Lakelse lake, showed a 98.6 $\pm$ 0.3% fertility in 14,827 excavated eggs.

The losses while emerging from the gravel and migrating downstream remain to be added to this total. Shifting gravel and silt deposition must create additional obstacles to the relatively delicate alevins. Undoubtedly stream predation is also a factor, but not an obvious one if the major spawning stream at Lakelse lake is typical. Few predator fish have been observed or caught in the streams and not more than one pair of kingfishers and two to three pairs of mergansers have been observed. These few would have to be exceptionally active to have much effect on a migration which is rapid in point of numbers moving from stream to lake. From an average seeding in Williams creek of 50,000,000 eggs (approximately 12,000 females) the majority of which are not over two miles from the lake, over 33,000,000 fry may be expected which must emerge and migrate at an average rate of about 1,000,000 per day. Although it has been shown by Cameron (MS.) that pink salmon experience heavy losses from predation during their migration as fry, the comparable reduction for sockeye appears to occur in the lake.

Fry to Migrant (within the lake). A number of agents might be listed as possible limiting factors in the lake survival of young sockeye: for example, disease, temperature, oxygen, food abundance, competition and predation. Of these, only the factors of food abundance, competition and predation can be accepted at present as being of importance at Lakelse lake or elsewhere in the Skeena river system. No data to support any other thesis have been obtained. It can be assumed that the ultimate limiting factor is one of food abundance. However this limitation would reduce individual size before it reduced numbers, an important consideration when dealing with a fish that moves to a new environment, one which is likely to have greater food abundance.

From stomach analyses predation by certain species of fish has been demonstrated as a significant agent affecting sockeye abundance.

Limiting factors are not usually believed to operate simultaneously but rather in the manner of a chemical chain. It might be supposed that predation, for example, was limiting the sockeye population of Lakelse lake in a natural, balanced state and that if predation were not present then food would become a limiting factor. Since animal populations are usually thought to be controlled by the lower links of food chains (Elton 1927) then it would seem exceptional for predators to become the controlling factor of prey. This is, however, quite possible for a food chain of a more complex nature than the simple direct predator-prey relation. Stomach content analyses of the squawfish, *Ptychocheilus*

oregonensis, (tables II and III) in Lakelse lake have revealed a sockeye content of approximately 31%, and a stickleback (*Gasterosteus aculeatus*) content of 28%. These two fish make up the bulk of the summer diet in some years and exhibit a wide variation in the extent of their contribution in different years. If the number of sticklebacks were suddenly reduced in Lakelse lake, the sockeye would

TABLE II. Variation in the total stomach content of squawfish at Lakelse lake.

Year	No. of stomachs	Netting period	Stomach contents (volume %)				
			Insecta	Mollusca	Cyclostomata	Pisces	Plant
1944	41	Jul.-Aug.	1.2	0.0	0.0	97.8	1.0
1945	202	Apr.-Aug.	5.8	2.4	2.6	84.2	1.4
1946	190	" "	14.1	2.6	0.0	76.7	6.6
1947	209	May-Sep.	22.8	0.0	5.6	68.5	3.1
Total	623	Average	10.2	1.9	2.1	83.1	3.2

suffer greater depredations from the predators. Conceivably fluctuations in the stickleback population might have an effect on the survival of sockeye parr through the link of predator fish. When it is pointed out that there are eleven species of fish, of both predators and competitors, present in Lakelse lake, the complexities of the predator relations with young sockeye become evident.

TABLE III. Variation in the identifiable fish content in the stomachs of 623 squawfish, Lakelse lake.

Year	Stomach content (volume %)			
	Sockeye	Stickleback	Peamouth	Sculpin
1944	2.3	68.0	29.7	0.0
1945	19.9	21.2	22.4	36.3
1946	75.4	17.3	0.0	7.4
1947	49.3	25.7	24.9	0.0
Average	31.3	27.7	19.2	21.8

Such postulations as the stickleback-squawfish-sockeye relationship are based on the assumption that predator fish actually do account for the losses recorded in the lacustrine stage of young sockeye. Continuous effort has been made at Lakelse lake to estimate this predation. It involves all the problems relative to estimating any fish population. Petersen's method of marking and recapture has been employed, with just over 2,500 fish of all species marked and released. The returns from these number 45. To calculate a possible population

on the basis of these recaptures without a knowledge of the differential marking mortality and natural mortality is hardly warranted. However, if an assumption were made that these mortalities accounted for 66% of the marked fish then the population of catchable fish (gill nets) would be in the region of 930,000 for all species. From the average catch per net-night (Withler, MS.) the predator fish constitute 26% of the total catches, which places then at about 240,000. The heavy losses encountered by sockeye fry from an average seeding could be accounted for by such a population of predators if the average consumption was only 140 per year, a figure which is quite conceivable.

DISCUSSION

For a balanced state in nature, from the spawning of one pair of sockeye salmon with an average egg content of about 3,900, two adults would be expected to return and spawn. The expected per cent survival from egg to spawning adult is therefore about 0.05, or one in 2,000. A high mortality is the natural state. The problem is that of locating where mortality assumes its greatest rate of numerical reduction.

At Cultus lake natural propagation resulted in different percentage survivals from egg to one year old migrant of 1.05, 1.13 and 3.23 (Foerster 1938a). A somewhat lower survival than this was calculated from the Karluk lake records, a figure of less than 1% being obtained for two year old migrants (Barnaby 1944). The Lakelse lake survivals are of the same order yet, like the Cultus lake records, show considerable variation. Lacking the accuracy of the Cultus lake parent enumeration further comparison does not seem warranted. It serves, however, to substantiate the evidence that about 98% of the original egg deposition is lost prior to seaward migration. Any slight alterations in this per cent survival will have marked effect on the total stock.

Only a long term study can sort out the interaction of the numerous variables which determine the number of sockeye surviving to leave the lake. Although predation has been labelled as the prime factor it is still a delicate balance of limiting factors which, if disturbed, must have sufficient follow-up to determine the actual path of changed survival. To decrease predation, only to be followed by a possible increase in competition in later years suggests the desirability of experimental areas to be used as test cases.

ACKNOWLEDGEMENTS

This study, under the direction of Dr. A. L. Pritchard, was a part of a programme of research conducted on the Skeena river, B.C., by the Pacific Biological Station. His constant encouragement and constructive suggestions have been greatly appreciated. Dr. R. E. Foerster has also added his critical advice and experience to forward the objectives of the investigation. Many others have contributed to the onerous task of maintaining the fence and counting the migrants. In particular Mr. D. F. Alderdice shouldered much of the latter responsibility.

SUMMARY

1. The survival from estimated sockeye egg deposition to seaward migrant has been calculated at 1.1% for 1946, 0.4% for 1947 and between 1% and 2% for limited data in 1948.

2. A sample of 1,267 eggs and alevins removed from seven redds showed an average survival of 87%, five months after the spawning period.

3. An average fish diet of 83% by volume was obtained in 623 squawfish stomachs. Young sockeye constituted 31% of the total fish content, with considerable annual variation.

4. The losses in young sockeye during lake residence at Lakelse could be accounted for by the predacious squawfish population.

REFERENCES

BARNABY, J. T. Fluctuations in abundance of red salmon (*Oncorhynchus nerka* Walbaum) of the Karluk river, Alaska. *Fish. Bull., U.S. Fish and Wildlife Serv.*, **50** (39), 237-295, 1944.

BRETT, J. R. The design and operation of a trap for the capture of migrating young sockeye salmon. *Trans. Am. Fish. Soc.*, **75** (1945), 97-104, 1948.

CAMERON, W. M. The efficiency of fertilization in natural spawning of Pacific salmon. *Prog. Rep. Pac. Biol. Stn.*, **45**, 23-24, 1940.

Mortality during the freshwater existence of the pink salmon. MS., Pac. Biol. Stn., Nanaimo, B.C., 1940.

ELTON, C. Animal ecology, 1-207. MacMillan Co., N.Y., 1927.

FOERSTER, R. E. An investigation of the relative efficiencies of natural and artificial propagation of sockeye salmon (*Oncorhynchus nerka*) at Cultus lake, British Columbia. *J. Fish. Res. Bd. Can.*, **4** (3), 151-161, 1938a.

Mortality trend among young sockeye salmon (*Oncorhynchus nerka*) during various stages of lake residence. *J. Fish. Res. Bd. Can.*, **4** (3), 184-191, 1938b.

NEAVE, F. Fecundity and mortality in Pacific salmon. Trans. Roy. Soc. Can., **17** (3), 97-105, 1948.

PRITCHARD, A. L. Fish cultural problems involved in the conservation of anadromous fish with particular reference to salmon on the Skeena river, British Columbia. *Can. Fish Cult.*, **1** (2), 8-13, 1947.

PRITCHARD, A. L. AND J. R. BRETT. A sockeye salmon tagging experiment at Lakelse lake. *Prog. Rep. Pac. Biol. Stn.*, **62**, 4-6, 1945.

PRITCHARD, A. L. AND W. M. CAMERON. Observations on the sockeye salmon run at Lakelse lake (Skeena river) in the year 1939. *Prog. Rep. Pac. Biol. Stn.*, **43**, 14-16, 1940.

WITHLER, F. C. Distribution, relative abundance and food habits of the fishes of the Skeena drainage with particular reference to predation and competition. MS., Pac. Biol. Stn., Nanaimo, B.C., 1948.

The Value of Temperatures Close to Freezing on the Storage of Fish

By C. H. Castell and W. A. MacCallum

ABSTRACT

It was observed that a reduction in storage temperature from 2.8°C. to − 0.3°C. approximately doubled the keeping time for haddock and cod fillets as judged by the development of trimethylamine. This reduction of 3° in the region of the freezing point added to the keeping time of commercial fillets approximately the same number of days as a reduction of 22° (from 25°C. to 2.8°C.) at a higher temperature range.

Somewhat similar results were obtained by reducing the storage temperature of whole, gutted codfish.

INTRODUCTION

The beneficial effect of reduced storage temperature on the keeping quality of fish is a long established fact. The special importance of the temperature range immediately above freezing was observed by Dreyer (1926) who followed the production of both trimethylamine and ammonia held at 0° to 1.7°, 7.2° to 10° and 16.7° to 18.3°C, and found that at the lowest range the production of both these compounds was very much retarded. Hess (1932) made a very thorough study of the influence of temperatures within the range of − 1.1 to 2.2°C. (30° to 36°F.) on the autolytic and bacterial decomposition of haddock. His results showed that bacterial decomposition at − 1.1°C. (30°F.) was about half of that at 2.2°C. (36°F.) and demonstrated the economic importance of holding fresh fish at 30°F. instead of the customary holding in crushed ice at about 36°F.

Although these earlier results of Hess were obtained by studying the changes occurring in extracts and emulsions of fish muscle, as well as in a few headed and gutted fish, the accuracy of his findings have been amply demonstrated by similar work applied in a more extensive scale under ordinary commercial conditions. The following experiments are of this nature and include work with fillets and round fish of cod and haddock.

EXPERIMENTAL METHODS

In the first experiments fillets of cod and haddock were used. Although the individual fillets in each lot of fish were as nearly alike as possible, an effort was made to include experiments with fish having a wide range in their initial quality. The fillets were either wrapped in waxed paper or stored in waxed cardboard boxes. The storage temperatures were − 0.3°C. (31.5°F.), 0.6°C. (33°F.), 2.8°C. (37°F.), and for a few tests 7.2°C. (45°F.) and 25°C. (77°F.).

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

Periodically, slices of approximately 50 to 60 grams were cut from each fillet in the lot being examined. These were macerated together in a Waring Blendor with one and a half times their weight of sterile water and used for the bacterial plate counts and for the determination of trimethylamine values.

The plates for the bacterial counts were incubated at 25° and 3°C. When the fish themselves were stored at 2.8° or lower, the counts on the plates at 25° were higher than those at 3°, for the first few days, but as the storage period of the fish continued, the counts at 25° and 3° became approximately the same. The nutrient agar used was that described by Dyer, Dyer and Snow (1946). Trimethylamine was measured by the method of Dyer (1945).

EXPERIMENTAL RESULTS

Fig. 1 gives the spoilage curves in terms of trimethylamine nitrogen for six lots of fillets stored at − 0.3°, 0.6° and 2.8°C. Judged on a basis of initial quality, these particular sets were picked as the two worst, the two best, and two typical intermediate lots of fillets from a larger group of experiments. If a trimethylamine value of 15 is taken as the point where the fillets first show definite signs of spoilage (Dyer and Dyer 1949), it is evident that a reduction in the storage temperature from 2.8° (37°F.) to − 0.3° (31.5°F.) had added from 1½ to 7 days to the keeping time of these particular fillets (table I). It is interesting to note

TABLE I. The number of days required for 7 lots of cod fillets to reach a trimethylamine value of 15 when stored at 2.8°, 0.6° and − 0.3°C.

Lot No.	Days at 2.8°C.	Days at 0.6°C.	Days at − 0.3°C.
1	7½	9½	13
2	5¼	7½	12
3	5	6½	8
4	3¾	4½	5¼
5	3½	4	4¼
6	3	4¼	5
7	1½	2	2¾

that regardless of the initial quality the keeping time at − 0.3° (31.5°F.) was roughly twice that of the corresponding lots of fish at 2.8° (37°F.).

In another group of 6 boxes of fillets, which represented a more normal selection from the cutting rooms of a local plant the approximate keeping times (up to trimethylamine value of 15) were as follows:—

	Storage Temperature	*Keeping Time*
Stored at	−0.3° (31.5°F.)	11-12 days
	0.6° (33°F.)	6-8 days
	2.8° (37°F.)	5-6 days
	7.2° (45°F.)	2-3 days
	25° (77°F.)	22-30 hours

These figures clearly stress the significance of the temperature range immediately above freezing. A reduction of 3° (from 2.8° to − 0.3°) adds to the keeping time of the fillets approximately the same number of days as a reduction of 22° (from 25° to 2.8°) at a higher temperature range.

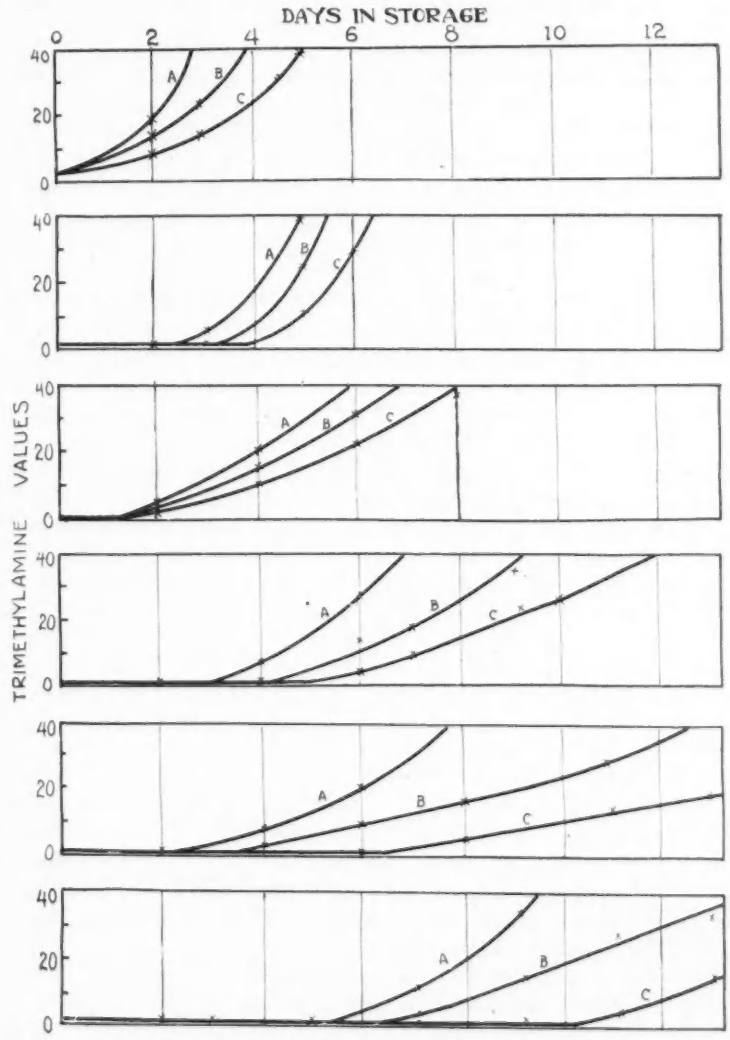


FIGURE 1. Spoilage rates for 6 lots of fillets, in terms of trimethylamine. Each group of fillets was divided into three parts; one part was incubated at 2.8° (A), another at 0.6° (B), and the third at − 0.3° (C). The fillets represented by the upper graphs were of a decidedly inferior quality at the start. Those at the bottom more nearly approached the normal commercial fillets as they leave the cutting tables in the fish plants.

ADDITION OF NITRITE

If the fillets are treated with sodium nitrite so as to contain 180 to 200 p.p.m. before storage the apparent effect of these small differences in storage temperatures becomes even more striking when examined in terms of trimethylamine (table II). However, this is not a true picture of what actually occurs. Bacterial

TABLE II. The number of days required for fillets of cod and haddock to reach a trimethylamine value of 15, with and without 180 to 200 p.p.m. sodium nitrite and stored at three different temperatures.

Storage temperatures	Days in storage to reach trimethylamine value of 15					
	Cod A		Cod B		Haddock	
	Control	+ NO_2	Control	+ NO_2	Control	+ NO_2
2.8°(37°F.)	3	7	5	9	6	> 20
0.6°(33°F.)	4	13	7	14	6	> 20
− 0.3°(31.5°F.)	6	17	12	21	8	> 20

counts, in these and similar experiments, showed that the bacteria increased at approximately the same rate in the treated and untreated fish. Although the trimethylamine production was suppressed in the nitrite treated fillets, other spoilage changes took place. This was particularly evident in the haddock fillets which had a trimethylamine value of 1.0 to 3.0 mg. nitrogen per 100 g. after 20 to 30 days storage (which would approximate a fresh condition in untreated fillets), although the bacterial counts were a billion or more per gram, and a definite putrid odour was present.

When the same fillets were judged by appearance and odour, the nitrite-treated fillets were invariably slower in developing off odours than the untreated ones at each of the different storage temperatures.

SPOILAGE OF WHOLE GUTTED FISH

The spoilage of round fish is caused by the same organisms that act on the fillets. In fact, the slime on the surface of the round fish is the chief source of the fillet contamination. One would suspect, therefore, that a similar temperature effect could be observed with the storage of the round fish as has been obtained with the fillets. That this is the case is shown by the following unpublished tests made by H. Pivnick and J. Richards at this station.

"Forty-nine market cod, three to four pounds in weight, one day on ice, were obtained from a local trawler. After determining the initial trimethylamine value, the fish were doubly wrapped in waxed paper and divided into four equal groups which were stored at 0°, 3°, 5° and 10°. At intervals three fish were removed from each group and the trimethylamine content of the nape, middle and tail muscles was determined. The mean time required for the middle muscle

to reach the spoilage threshold of 15 mg. of trimethylamine at each of the temperatures was as follows:—

Storage Temperature	*Keeping Time*
0°C.	8.1 days
3°C.	4.9 days
5°C.	3.4 days
10°C.	1.5 days

"The nape muscle spoiled at about the same rate, and the tail muscle a little more slowly."

A somewhat similar experiment, also using a trimethylamine value of 15 as the threshold of spoilage, gave the following results:—

Storage Temperature	*Keeping Time*
0°C.	8.0 days
3°C.	4.3 days
10°C.	1.5 days

From this it can be seen that as with the fillets, so with the round fish, a reduction in the storage temperature of three degrees immediately above freezing adds proportionally far more to the keeping time than a greater reduction at a higher range.

DISCUSSION

The spoilage rate for stored, unfrozen fillets is governed by a number of different factors. Of these, the first, the number and types of bacteria, and especially the number of the gram negative psychrophilic species, has been discussed in a previous paper (Castell *et al.*, 1948). The second important factor is that of the temperature at which the fillets are held. Of less importance but still affecting the keeping time of the fish are: the species of fish, the physical and chemical changes that have occurred in the muscle up to the time of storage, and the use of preservatives such as nitrite.

The results in the tests recorded in this paper confirm the earlier observations of Hess (1932) in emphasizing the importance of slight differences in the storage temperature for fresh fish when the range is within 5 degrees of freezing. With most of the commercial fillets that were tested, a difference of 3.1° (5.5°F.) approximately doubled the time for the trimethylamine value to reach 15. It is also very significant that this relationship apparently holds, regardless of the spoilage rate at the higher temperature.

Somewhat similar results were obtained by Dyer and Dyer (1949) in their study of palatability changes of stored cod fillets. They observed that fillets which became unacceptable after 3 days storage at 41°F. (5°C.) reached the same condition when stored at 32°F. (0°C.) for 8 days.

It is interesting, and from a practical standpoint very significant, to see that a reduction of 3 degrees, close to the freezing point (from 2.8° to − 0.3°) added to the keeping time of both fillets and round fish approximately the same number of days as a reduction of 22 degrees (from 25° to 2.8°) at a higher temperature range.

In other words, a fish plant producing a quality of fillet that will keep for 2 days at 2.8° (37°F.) can increase the keeping time 2 more days by reducing the storage to − 0.3° (31.5°F.). However, if by increased sanitation and care of the fillets they so improve the initial quality of the fillets that they will last for 6 or 7 days at 2.8°, they can then add another 6 or 7 days by a small reduction in storage temperatures. The value of reducing the storage temperature depends upon the initial quality of the fillets.

Similarly, the storage time of the round fish in the vessels at sea can be greatly lengthened and the quality at the time of landing can be improved if all the fish were kept as near as possible to 0°C. When portions of the hold are permitted to warm up to 5°C. or even 3°C. we can expect the fish at those points to spoil from 4 to 5 days sooner. At 10°C. it is only a matter of a day or so before the spoilage sets in. These results appear to be confirmed by the improved quality of the fish landed in the local fishing vessels in which ice is supplemented by refrigeration to keep the entire catch as near the freezing point as possible.

Because of the uselessness of the trimethylamine value as a measure of spoilage in nitrite treated fish, no measured data was obtained to show the additive effect of reduced storage temperature and nitrite. However, that such a result can be obtained was evident from the organoleptic examinations.

If a fish plant operator finds his market limited by the relatively short keeping time of his fillets, there are three things that he can do: (1) strive to produce fillets with a better initial quality, (2) reduce the storage temperature to as near freezing (0°C.) as possible or even a degree or so below, (3) supplement these two procedures by the careful use of nitrite dip.

REFERENCES

Castell, C. H., G. W. Anderson and H. Pivnick. *J. Fish. Res. Bd. Can.*, **7** (6), 378-388, 1948.

Dyer, W. J., F. E. Dyer and J. M. Snow. *J. Fish. Res. Bd. Can.*, **6** (6), 403-413, 1946.

Dyer, F. E. and W. J. Dyer. *J. Fish. Res. Bd. Can.* **7** (8), 449-460, 1949.

Dyer, W. J. *J. Fish. Res. Bd. Can.*, **6** (6), 351-358, 1945.

Dreyer, N. B. Bio. Bd. Can. M.S. Repts. Expt. Stas. 11 (unpublished).

Hess, E. *Contrib. Can. Biol.*, **7**, 147-163, 1932.

Some Observations Relating to an Unidentified Growth Factor for Young Salmonoid Fishes. I.

By Norman E. Cooke and R. V. Tomlinson
Pacific Fisheries Experimental Station

(*Received for publication January 12, 1950*)

ABSTRACT

The four major components of salmon viscera were tested as food for young trout. It was found that there is a growth-promoting factor in the roe and the milt which is not contained appreciably in beef liver, salmon liver, or salmon digestive tract. It was found also that this factor could be extracted from the roe both by water and by methyl alcohol, but not by petroleum ether.

INTRODUCTION

As was recently pointed out by Karrick (1948), fish require many unknown nutritional factors and "it has not been possible to rear fish for any length of time on a diet consisting of substances with a definitely known composition." While this recent review precludes any necessity for an extensive survey of the literature by the present authors, it should be mentioned that over twenty years ago, a group of workers at Cornell University pointed out that trout need fresh meat, or some substance contained in it, in order to live and grow properly (McCay and Dilley 1947; McCay, Bing and Dilley 1928). This substance, called Factor H, was shown to be different from any vitamin known at that time. Since that early work, there have been several attempts to identify this factor. Hewitt (1936) suggested that a mixture of phosphotides was responsible. Tunison *et al.* (1943) claimed that a mixture of pantothenic acid, riboflavin and pyridoxine was the factor; but a year later, this same group of workers was unable to confirm its work (Tunison *et al.* 1944). Norris and Simmons (1941) claimed that injections of xanthopertin cured the anemia associated with the lack of Factor H. Xanthopertin, however, was found by Tunison *et al.* (1947) to be ineffective when mixed with the food. McLaren *et al.* (1947b) have claimed that folic acid and/or biotin are specific for curing the associated anemia but, while this claim has been neither confirmed nor disproved, it is significant to note that the basic diet used in these experiments contained 8% acid-treated crab meal which was shown to contain some unidentified factor which promoted growth (McLaren *et al.* 1947a).

The recent work of Burrows and Karrick (1947) re-emphasized the value of salmon viscera in the nutrition of young salmon and indicated that the viscera contained some factor over and above those contained in beef liver. Since

J. Fish. Res. Bd. Can., 8 (2) 1950.
Printed in Canada.

considerable work on the utilization of salmon waste is being carried out at this station, it was decided to investigate this possible use of the viscera. At the same time, however, it was decided to approach the subject from as fundamental a standpoint as possible, and to attempt the isolation and/or the identification of the factor or factors involved.

This paper reports on two experiments carried out in the fall, winter and spring of 1948-9. The first experiment was designed to ascertain which of the components of the viscera contained the factor. The second experiment was based on the principle that, since the yolk sac of the egg is designed to be the sole source of food for the young fish during a critical period of their lives, the eggs should contain all the factors which are essential for proper growth. The diets used in these experiments are outlined below.

EXPERIMENTS

I. The Diets and Their Preparation

As has been stated, the first experiment was to find out which parts of the viscera contain the factor or factors concerned. Six diets, as outlined in table I, were used.

Table I. Composition of diets in first experiment.

Diet number	Component A (55%)	Component B (30%)	Component C (15%)
1	Raw beef liver	Raw beef liver	Dried beef liver
2	Raw beef liver	Raw salmon roe	Dried salmon roe
3	Raw beef liver	Raw salmon milt	Dried salmon milt
4	Raw beef liver	Raw salmon digestive tract	Dried salmon digestive tract
5	Raw beef liver	Raw salmon liver	Dried salmon liver
6	Raw beef liver	Raw mixed salmon viscera	Dried mixed salmon viscera

These diets consisted of a basic amount of raw beef liver with the addition of the material to be tested in both the raw and dried states. The dry material was added to give the diet a good consistency for proper feeding. It was made by coagulating the raw material in a retort at 10 pounds (gauge) steam pressure for 20 minutes, grinding, spreading on trays and drying in a hot air tunnel at 140° F. (65.6° C.). The material was ground several times during the drying to speed up the process. When the material was dry, it was ground to pass through a 20-mesh screen.

Mixing the components into a diet was done in a commercial meat chopping machine known as a "silent cutter". The diets had the consistency of a heavy porridge. They were packed in one-pound cardboard cartons, and stored in a refrigerated locker until they were used.

The second experiment employed eight diets, arranged in pairs; the extract in one diet, the residue in the other. The composition of these diets are best explained by table II.

TABLE II. Composition of diets in second experiment.

Diet number	Protein source	Carbohydrates	Fat	Mineral mixture	Vitamin source	Water	Filler
7	a_1	b	c_1	d	e	f_1	g
8	a_2	b	$c_2 + c_3$	d	e	f_2	g
9	a_1	b	c_1	d	e	f_2	g
10	a_2	b	$c_2 + c_3$	d	e	f_1	g
11	a_2	b	$c_1 + c_3$	d	e	f_1	g
12	a_1	b	c_2	d	e	f_1	g
13	a_2	b	c_2	d	e	f_1	g
14	a_1	b	$c_1 + c_3$	d	e	f_1	g

The significance of the letters is as follows:

Protein source: a_1 = 20% dried skim milk
a_2 = 20% salmon egg protein
Carbohydrates: b = 20% starch
Fat source: c_1 = 5% lard
c_2 = salmon egg petroleum ether extract (oil)
c_3 = salmon egg methyl alcohol extract (phospholipids)
Minerals: d = 2% salts (Hubbell *et al.* 1937)
Vitamin source: e = 5% yeast + 25 grams choline per 100 pounds of diet
Water: f_1 = 32% tap water
f_2 = 32% water used to extract or contained in salmon eggs
Filler: g = 16% wood flour

Although the basic diet (diet 7) is not ideal, it proved satisfactory for the purposes of the experiment. In any further work, it is planned to use a diet with a purer source of protein and a more exact source of the vitamins. The diets in this experiment were mixed and stored in the same manner as described above.

II. The Mechanics of the Experiment

The experimental animals were fry of the species *Salmo gairdneri kamloops* Jordan. The experiment was started with 750 fry in each of the 14 troughs. Weighing was carried out at two-week intervals; ten lots of ten fish from each trough were weighed. By this method it was possible to find the mean weight of the sample taken and also to calculate the standard deviation about the mean.

The fish were fed approximately six per cent of their body weight of food per day in two lots. In the cases in which the fish grew poorly this was in excess of the amount the fish ate, on the other hand, the more rapidly growing fish usually ate all the available food.

III. Statistical Methods Used in the Treatment of Results

Some statistical methods used for interpreting the results have not been used previously, to the authors' knowledge, in the interpretation of the results of experiments of this kind. The Whittaker-Henderson method of graduation is extensively used, however, in the actuarial field.

As mentioned, ten lots of ten fish each from each trough were weighed every two weeks.

For simplicity of weighing and calculating, ten fish constituted a unit.

From the ten weights, two values were calculated:

$$\text{the mean: } \bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

$$\text{and the variance: } s^2 = \frac{1}{n}\sum_{i=1}^{n} x_i^2 - \bar{x}^2 \ .$$

In order to obtain a smooth curve to represent the mean over the whole experiment, the $\bar{x}$'s were graduated using the Whittaker-Henderson graduation Formula A after the method of Spoerl (1937). With the tables given by Spoerl, this method is exceedingly simple.

Confidence limits on the graduated means $\bar{x}_g$ were obtained in the following manner:

s^2 is the second moment about the mean $\bar{x}$.

s_g^2, the second moment about the graduated mean, $\bar{x}_g$ is desired. It follows from the parallel axis theorem and the definition of a frequency function that: $s_g^2 = s^2 + d^2$ where d is the distance between $\bar{x}$ and $\bar{x}_g$. Now it is desired to combine the several s_g^2 to obtain the best estimate of the variance. If the variances are equal this is:

$$\hat{\sigma}_g^2 = \frac{n_1 s_{g1}^2 + n_2 s_{g2}^2 + n_3 s_{g3}^2 \ldots\ldots n_k s_{gk}^2}{n_1 + n_2 + n_3 \ldots\ldots n_k - k} \ .$$

Since in this case, $n_1 = n_2 = n_3 = \ldots . n_k = n$

$$\hat{\sigma}_g^2 = \frac{n\sum_{i=1}^{k} s_{gi}^2}{kn-k} = \frac{n}{k(n-1)}\left\{\sum_{i=1}^{k} s_i^2 + \sum_{i=1}^{k} d_i^2\right\}$$

$\hat{\sigma}_g^2$ is the estimate of variance of the unit of ten about the graduated mean $\bar{x}_g$, but the variance of the mean itself is desired:

$$\hat{\sigma}_{\bar{x}_g}^2 = \frac{\hat{\sigma}_g^2}{kn}$$

$$\hat{\sigma}_{\bar{x}_g}^2 = \frac{\sum_{i=1}^{k} s_i^2 + \sum_{i=1}^{k} d_i^2}{k^2(n-1)} \ .$$

Since the number of observations involved is large (130), the confidence limits on the mean can be calculated from normal distribution tables. Confidence limits on the mean of 99% will therefore be

$$\bar{x}_g \pm 2.57\, \hat{\sigma}_{\bar{x}_g}$$

or

$$\bar{x}_g \pm \frac{2.57}{k} \sqrt{\frac{\sum_{i=1}^{k} s_i^2 + \sum_{i=1}^{k} d_i^2}{(n-1)}} \,.$$

In the particular case at hand, when $k = 13$ and $n = 10$ the expression becomes

$$\bar{x}_g \pm 0.0659 \sqrt{\sum_{i=1}^{13} s_i^2 + \sum_{i=1}^{13} d_i^2} \,.$$

The graphs in fig. 1 are constructed on the procedure outlined above, the heavy line on the graph representing the graduated mean, and the two dotted lines associated with the heavy line representing the 99% confidence limits. The graphs in fig. 2 were calculated in the same manner, but for the sake of clarity the confidence limits were omitted in the figure. In the cases of diets 7

FIGURE 1

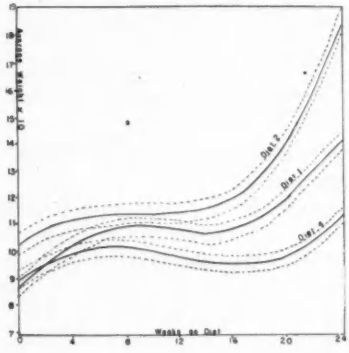
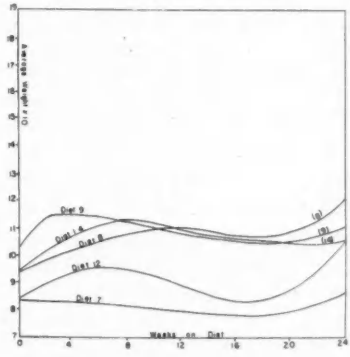


FIGURE 2



and 12 the mortality near the end of the experiment was excessive and also very selective; the smaller fish died in such great numbers at this time that only the large fish were left. This, of course, forced the mean to be erroneously high. To overcome this factor to some extent, the graduation in these two cases was calculated only on the first twelve values instead of the full thirteen.

Statistical comparison of the mortality data of any two diets was accomplished by testing the mean-weekly-difference of the number of deaths of the fish on the two diets by means of Student's "t" test.

RESULTS

The results of the first experiment were not quite those which would have been predicted before the experiment started. In general, the growth from these first six diets fell into three groups: those better than the control (diets 2, 3 and 6), those the same as the control (diet 5) and those worse than the control (diet 4). Fig. 1 shows the growth curves for diets 1, 2 and 4. The other growth curves are omitted for sake of simplicity.

Somogyi (1949) has shown that trout viscera (presumably the digestive tract) contains appreciable amounts of thiaminase and there is every likelihood that this factor is responsible for the depression of growth of the fish on diet 4.

While none of the diets used in the second experiment produced growth as good as the beef liver control, it is felt that this is largely only a matter of the amount of the components fed. Further work will clarify this point. Fig. 2 shows the growth curves of the fish on diets 7, 8, 9, 12 and 14. From a study of these curves and table II it becomes apparent that the growth factor or factors can be extracted from the roe by water, and by methyl alcohol but not by petroleum ether. These growth curves are substantiated completely by the mortality data. Diets 7 and 12 produced mortalities (99% confidence) greater than any of the other diets. One very interesting fact to note in this connection is that while diet 2, with a large amount of whole salmon eggs in it, produced a significant increase in mortality, diet 14 containing an extract of salmon eggs, produced only a very slight mortality.

CONCLUSIONS

(1) Salmon viscera contains a factor (or factors) which promotes the growth of young trout, and this factor is more particularly concentrated in the roe and the milt but not in the digestive tract or the liver.

(2) The digestive tract of salmon contains some factor which depresses the growth of young trout and causes a high mortality.

(3) The growth factor can be extracted from the roe by water or by methyl alcohol but not by petroleum ether.

(4) The methyl alcohol extract of the roe does not contain appreciable amounts of the factor which causes a high mortality.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with thanks the facilities extended to them by the British Columbia Game Commission who gave them full use of their hatchery at Cultus Lake, B.C. In particular they wish to thank Mr. Frank Pells without whose help in feeding and caring for the fish this experiment could never have been carried out.

Appreciation and thanks are also extended to Dr. W. A. Clemens and Dr. W. Hoar of the Department of Zoology and Dr. S. A. Jennings of the Depart-

ment of Mathematics of the University of British Columbia for their interest and suggestions concerning this work.

The help of Misses Iris Smith and Enid Young, technicians at this station, in working up the results and the additional assistance of the former in preparation of the diets is also greatly appreciated.

REFERENCES

BURROWS, R. E. AND N. L. KARRICK. Utilization of Alaskan Salmon Cannery Waste, *U.S. Dept. of Commerce, Industrial Research and Development Division, Washington, D.C.*, Report 1, 71-89, 1947.

HEWITT, E. R. *Trans. Am. Fish. Soc.*, **66**, 291-297, 1936.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN. *J. Nutrition*, **14**, 273-85, 1937.

KARRICK, N. *U.S. Dept. of Interior, U.S. Fish and Wildlife Service Fishery Leaflet*, **325**, 1-23, 1948.

MCCAY, C. M. AND W. E. DILLEY. *Trans. Am. Fish. Soc.*, **57**, 250-260, 1927.

MCCAY, C. M., F. C. BING AND W. E. DILLEY. *Science*, 67, 249-50, 1928.

MCLAREN, B. A., E. KELLER, D. J. O'DONNELL AND C. A. ELVEHJEM.
Arch. Biochem., **15**, 169-178, 1947a.
Arch. Biochem., **15**, 179-185, 1947b.

NORRIS, E. R. AND R. W. SIMMONS. *J. Biol. Chem.*, **158**, 449-53, 1945.

SOMOGYI, J. C. *First International Congress of Biochemistry Cambridge.* Abstract No. 441/1, August 19-25, 1949.

SPOERL, C. A. *Trans. Actuarial Soc. Am.*, **38**, 403-61, 1937.

TUNISON, A. V., D. R. BROCKWAY, J. M. MAXWELL, A. L. DORR, AND C. M. MCCAY. *Fish. Res. Bull. No.* **4**, *N.Y. State Conservation Dept., Bur. Fish. Culture, Albany, N.Y.*
Cortland Hatchery Report No. **11**, 1-52, 1942.

TUNISON, A. V., D. R. BROCKWAY, H. B. SHAFFER, J. M. MAXWELL, C. M. MCCAY, C. E. PALM, AND D. A. WEBSTER. *Fish. Res. Bull. No.* **5**, *N.Y. State Conservation Dept., Bur. Fish. Culture, Albany, N.Y.*
Cortland Hatchery Report No. **12**, 1-26, 1943.

TUNISON, A. V., A. M. PHILLIPS, H. B. SHAFFER, J. M. MAXWELL, D. R. BROCKWAY, AND C. M. MCCAY. *Fish. Res. Bull. No.* **6**, *N.Y. State Conservation Dept., Bur. Fish. Culture, Albany, N.Y.*
Cortland Hatchery Report No. **13**, 1-21, 1944.